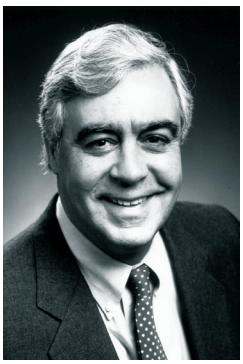


ACA Living History – Charles E. Bugg

In his memoir Charlie describes how an academic crystallographer reinvented himself as the CEO of a biotechnology firm. The company he founded, BioCryst Pharmaceuticals, applies structure-based drug design to invent drugs for cancer, gout, Marburg, Ebola, influenza, and hereditary angioedema. During his career he assumed a leadership role in the NASA efforts to grow protein crystals in space. He also was President of the ACA (1987) and Editor-in-Chief of *Acta Crystallographica* (1987-1996).

I was supposed to be an orthopedic surgeon, not a crystallographer. My father was a prominent orthopedic surgeon. He had a private practice and was on the faculty in Orthopedics at Duke in Durham, North Carolina, where I was born and raised. My mother handled the finances for his practice. I was also destined to attend Duke University, where both of my parents, my grandfather and multiple other relatives attended college. My father's number one recreation, which also became mine, was hunting and fishing. These were very productive activities in rural North Carolina back then. It was a wonderful time for me to grow up in the South.

My mother was a strong influence in my life from the earliest times I can remember. I initially attended Calvert School, now renamed Durham Academy, a private school where all of my close friends were enrolled. However, my mother was a strong advocate of public schools, and she served a number of years on the Durham School Board. Although I think my family could have afforded private school at the time, she moved me to Morehead School, a public elementary school, when I was in the fourth grade. This school was in a pretty rough neighborhood. It seemed that I was routinely roughed up every day after school, and I made it clear that I thought I really should return to Calvert. My mom's solution was to hire a retired professional boxer to give me lessons in how to take care of myself. She sent me back into the jungle, where I finished elementary school. I actually ended up making some very good friends there, who had interesting backgrounds that I would have totally missed if I had stayed in private school.

Academic crystallography. I was admitted to Duke as a pre-med student in the summer of 1959. A real stroke of good luck was meeting Bebe Bradshaw on the first day of freshman orientation. She was and is my soul mate and has been a key support and driving force in all aspects of my life and career since those early years at Duke. My goal of becoming an orthopedic surgeon was gradually replaced by my interest in science; I really was turned on by physical chemistry, thanks to a superb professor, Marcus Hobbs. Professor Hobbs arranged for me to be admitted to the Rice graduate program. There I was fortunate to be accepted as a student in the laboratory of Ronald Sass, a young, dynamic faculty member pursuing various research programs in crystallography. I quickly became an expert in Weissenberg photography and manually estimated the intensities of thousands of film spots by comparing each separately with diffraction spots produced on standardized filmstrips. Computing was also a major challenge at the time, but it was fortunate that the Department of Electrical Engineering at Rice had recently constructed a computer that was available at night and on weekends. This computer occupied a complete floor of the engineering school and was constantly breaking down. It probably had a tiny fraction of the power of a modern smartphone, but it beat calculating Fourier maps by hand. When my PhD thesis was completed in 1965 I did not know exactly what I wanted to do with the rest of my life. Philip Handler, the Chairman of Biochemistry at Duke, was charismatic, knowledgeable and persuasive in his view that crystallography was a wonderful opportunity for me in biology. With help from Dr. Sass, a postdoctoral position was arranged at Caltech, in the laboratory of Dick Marsh and Bob Corey, and I joined them in the spring of 1965.

At Caltech my crystallography training moved to an entirely new level under the supervision of Dick Marsh. Dick is a notorious stickler for high precision in all aspects of crystallographic structural studies, beginning with collection of accurate diffraction data and through the final writing of a proper manuscript describing the analysis and results. I like to think that much of his obsession with doing everything as perfectly as possible rubbed off on me during my time with him, and that I, in turn, have had some success in passing those principles on to my students and postdoctoral fellows. Following the Watson-Crick discovery of the double helical structure of DNA, there was broad interest in better understanding the detailed atomic-level structures of nucleic acid components so that more precise models of nucleic acids could be developed. I was fortunate to obtain crystals of cytidylic acid, one of the four components of RNA, and the crystallographic analysis of that nucleotide became my first major project at Caltech. This also began what eventually became a multi-year career in crystallographic studies of nucleic acid components and their analogs.

The 1960's were a great time to be in science, and many career opportunities were available. I interviewed with several chemical companies and was especially excited by the broad research programs at DuPont. I ended up accepting a position with their polymer fiber division, at their research laboratories located in Kinston, North Carolina. Within six months, however, it was clear to me that a large company, even one as outstanding as DuPont, was not where I wanted to spend the rest of my life. I greatly missed the freedom and stimulation of academia. I submitted an application to NIH for a postdoctoral fellowship to continue my studies of nucleic acid components. I was delighted when I was awarded the fellowship and fortunately Dick Marsh was happy to accept me back into his lab.

In 1968 an unusual opportunity fell into my lap. The University of Alabama in Birmingham (UAB) had received a large NIH grant to establish an interdisciplinary Institute of Dental Research in Birmingham, which was home to one of the top dental schools in the country. I accepted positions as Assistant Professor in the Department of Biochemistry, Investigator in the Institute of

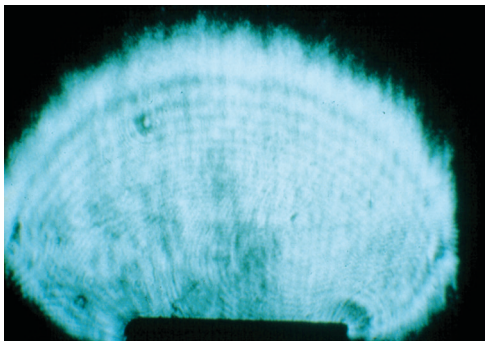
Dental Research, and Investigator in the Laboratory of Molecular Biology. I was extremely fortunate to be joined by my Caltech colleague Ulf Thewalt, who was eager to continue the fruitful crystallographic collaboration we had initiated in Pasadena. Our crystallography group undertook a variety of structural studies of purine and pyrimidine derivatives along with other molecules of biological interest. We also initiated productive studies of calcium and phosphate complexes and compounds, much to the joy of my colleagues in the dental field. I also enjoyed the benefit of collaborating with another of my Caltech colleagues, Mani Subramanian, who joined my group shortly after Ulf departed for a new faculty position in Germany. I think that these structural studies added significantly to the foundation for understanding the base stacking interactions of natural and modified purines and pyrimidines and the interactions that occur in biological systems between calcium and phosphate ions and various biological ligands. Howard Einspahr did a particularly beautiful job bringing together data from all of our calcium structures with other data from the Cambridge Structural Database to lay out a comprehensive picture of how calcium ions interact with various biological ligands.

In 1971, the UAB Cancer Center was designated one of the first Comprehensive Cancer Centers by the National Cancer Institute, and I served as the first Associate Director for Basic Sciences in the Center. We had an especially productive collaboration at that time with John Montgomery, a prominent medicinal chemist at nearby Southern Research Institute (SRI), and he was constantly urging me to focus our crystallographic studies on some of the important protein targets in cancer. It became increasingly clear to me that we needed to expand our Birmingham program into protein crystallography if we were going to take full advantage of opportunities in our new Cancer Center. UAB had a policy of optional faculty sabbaticals every seven years, and I decided to use this opportunity to learn the essentials of protein crystallography.

Sabbatical in Oxford. So, in the spring of 1974, Bebe packed up our three young children, and we took off for Oxford. My lab at Oxford was located next door to Dorothy Hodgkin, who had received the 1964 Nobel Prize in Chemistry for the structures of penicillin and vitamin B_{12}. She had transitioned to proteins and was then working on the structure of insulin. I was immediately at home and comfortable with Dorothy, who was incredibly warm and welcoming, and I felt that we shared a common bond in transitioning from small-molecule crystallography to protein crystallography. I quickly joined Margaret Adams on her studies of the enzyme 6-phosphogluconate dehydrogenase. Margaret was still in the early stages of determining this crystal structure, and she enthusiastically invited me to join her on this project. She proved to be a wonderful teacher who spent countless hours with me on details of protein crystallography. Margaret also provided me with another lifelong benefit when she introduced me to John Helliwell, a bright and enthusiastic graduate student working on this crystallographic project. John was at the early stage of his graduate research, so we were pretty much on the same level in our protein crystallography training and we were able to fully share the learning experience. We became close friends and continued to collaborate over the years after we left Oxford.

The PNP project. Shortly after my return from sabbatical in Oxford, John Montgomery and I undertook a project that would eventually cover many years of our future careers. We selected the human enzyme purine nucleoside phosphorylase (PNP) for pursuing structure-based drug design guided by protein crystallography. PNP had been demonstrated to be essential for normal immune responses since children born with defects in the gene for PNP lacked T-cell immunity. Inhibitors of PNP might prove useful clinically for treating T-cell mediated diseases, including a variety of autoimmune diseases, T-cell leukemias, and T-cell lymphomas. In addition, inhibition of PNP would block the biological synthesis of guanine from guanosine and could thus be used to inhibit the synthesis of uric acid, for treatment of gout. We knew that it would be a long and difficult road through the crystallographic studies, and through the eventual design, synthesis and development of inhibitors. Thus it was encouraging to have a target that might lead to drugs with multiple potential applications.

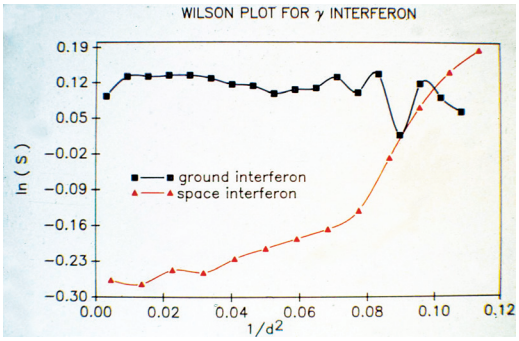
At this stage, John Helliwell had completed his doctoral studies and moved to Daresbury in northern England where one of the newly constructed synchrotron facilities was available. John had developed a beam line for X-ray crystallography, and he was delighted to join us as a collaborator on the structural studies of PNP. Bill Cook crystallized the enzyme and Steve Ealick led all of the crystallographic studies of PNP and of multiple complexes of the enzyme, work which encompassed much of the period between 1981 and 1985. The crystallographic analysis was a fairly difficult undertaking at the time since the crystals had a very high 80% solvent content, and thus diffracted relatively weakly.



A triglycine sulfate crystal growing in space with growing crystal face at the bottom. The disruptive density-driven convective flow seen on Earth is essentially eliminated in microgravity. This results in a more uniform growth process, which is governed by the rate of solute diffusion from the solution to the growing crystal surface. (Courtesy of Marshall Spaceflight Center.)

Crystallization in space. In 1985, our crystallography program at UAB took an unusual turn toward space. NASA was in the midst of designing the Space Station, and much of this work was being coordinated at the Marshall Space Flight Center in Huntsville, Alabama. Larry DeLucas developed into a charismatic leader of our space efforts, in collaboration with multiple NASA colleagues. By 1994 we had performed experiments on sixteen Shuttle flights. A total of 81 different proteins, provided by some 40 collaborators from protein crystallography groups around the

world, were included in crystal growth experiments. The most encouraging results were obtained in the space experiments with proteins that had been studied extensively, with successful crystallization results already obtained on Earth. Among this subset of proteins, there were several striking examples of improved crystal order as evidenced by enhanced diffraction resolutions and reproducible data from relative Wilson plots. At the time of this writing a huge set of double-blinded protein crystal growth experiments has just recently been returned from the Space Station for analysis by Larry and his collaborators, to evaluate the long-range potential of microgravity protein crystal growth.

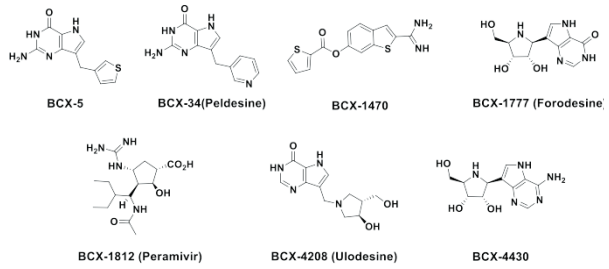


Relative Wilson plots comparing crystals of gamma interferon. Earth-grown crystals (black) are similar; the slope is zero. Space-grown crystals compared with Earth-grown crystals (red) are more highly ordered, giving a sloping line.

Service to ACA and *Acta*. In 1987, I had the pleasure of serving as the President of the American Crystallographic Association, and I decided to focus on the future of protein crystallography for my after-dinner talk the following year at the Philadelphia ACA meeting. I showed plots of the past growth of the Cambridge Structural Database and of the current growth rate of the Brookhaven Protein Data Bank, and I suggested that the plots overlaid pretty nicely when comparing the early stages of small-molecule crystallography with the then current growth rate for new protein crystal structures. If we assumed that the two growth functions were going to be approximately the same, I suggested that we could reasonably expect thousands of new protein crystal structures to be forthcoming during the next few years. This suggestion was met with considerable skepticism from my colleagues, but the Brookhaven Protein Data Bank soon saw a dramatic increase in the number of deposited structures. I later served as Chairman of the Brookhaven Protein Data Bank Advisory Board, which gave me an opportunity to help campaign for the increased funding that would be required for the Data Bank to handle the huge influx of new data. The last time I looked, the Protein Data Bank has data for well over 100,000 protein structures and is still growing rapidly. I also had the pleasure of serving as Editor-in-Chief of *Acta Crystallographica* and chairing the IUCr Commission on Journals during the 1987-1996 period. After much discussion with the protein crystallography community, and with the enthusiastic support of André Authier, President of the IUCr at the time, we initiated *Acta Crystallographica*, Section D, titled "*Biological Crystallography*," which is now one of the most popular journals in the *Acta* family.

Structure-based drug design. During the late 1980's, our crystallography group at UAB became increasingly focused on structure-based drug design, and we initiated crystallographic studies of several additional enzymes that we felt would be especially suitable drug design targets, including influenza neuraminidase and complement proteins. Both of these programs were later licensed from UAB to BioCryst. UAB was also focused on new approaches to molecular modeling that might be of broad use in structure-based drug design. Mike Carson led a creative modeling program focused on novel approaches for displaying protein sites by computer graphics in ways that would allow non-crystallographers to see features that would be helpful in drug design. Mike's early work produced the now popular algorithm for ribbon representation of polypeptide chains, and he designed new ways of displaying and interacting with protein sites. Scott Rowland pioneered other creative approaches for predicting interaction patterns that might be applied to drug design through extensive analysis of intermolecular contacts found in small molecule crystal structures from the Cambridge Structural Database.

BioCryst Pharmaceuticals. In 1985 we began to think seriously about seeking funding from private sources. BioCryst Pharmaceuticals, Inc. was incorporated in 1986. Y. S. Babu became our first employee, which turned out to be one of the most productive recruitments I ever made in my career. By 1993, our BioCryst/Ciba Geigy/UAB/SRI collaboration had produced a series of potent inhibitors of human PNP and a lead candidate, BCX-34 (later assigned the generic name peldesine) had been selected for clinical development by BioCryst. A second PNP inhibitor, BCX-5, was partnered with Warner Lambert Pharmaceutical Company for clinical development. When John Montgomery and I originally selected the PNP target for drug design back in the late 1970's, the objective was to end up with drugs for treating patients, so we were finally at an important milestone.



Some of the BioCryst compounds that have reached advanced stages of development.

The challenge we faced at that stage was to come up with the funds necessary to move BCX-34 forward into clinical development. I ended up grossly underestimating how much it would eventually cost to develop a PNP inhibitor, but it was clear that we would need to raise a lot of money to even initiate clinical development properly. Between 1986, when we first incorporated BioCryst, and 1993, we had repeatedly gone back to our original investors to raise additional funds. We had also brought in funding from a couple of prominent venture capitalists from national investment firms. However, these investors were not willing to

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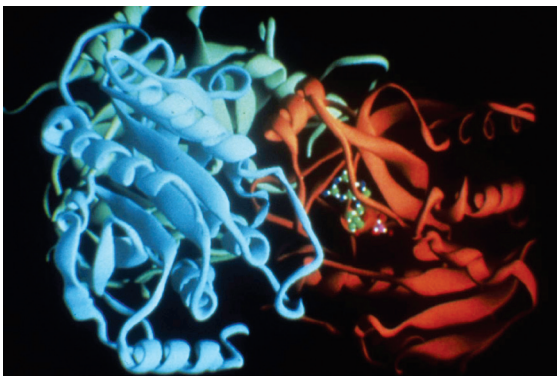
undertake the complete costs that would be required for clinical development of BCX-34, along with our planned expanded program for attacking additional targets. Our investors were painfully aware that drug development is incredibly expensive, very risky with high failure rates, and takes a long time to complete the necessary clinical trials for drug approval by the FDA. It was going to take a lot of capital, available continuously over a number of years, if we were to realize the goal of making our PNP inhibitors and other compounds available for treating patients.

The ideal strategy for us was to take BioCryst public through an initial public offering (IPO) of stock in the company. The bankers, analysts and the major investors involved felt that it would be critical for me to leave UAB and go fulltime with BioCryst. Bebe probably would have vetoed the move if Penny Mann, my wonderfully proficient administrative assistant at UAB, had not agreed to leave the university and come along to keep me organized, but fortunately Penny did. So on January 1, 1994, I jumped from my secure academic nest into the corporate world of biotechnology. It was immediately clear that I had a lot to learn, and I needed to learn it quickly. We successfully completed our IPO on March 7, 1994 and initiated trading on the NASDAQ stock exchange under the stock symbol BCRX.

Drugs for cancer, gout, Marburg, Ebola, influenza, hereditary angioedema. Meanwhile, Vern Schramm and his colleagues at the Albert Einstein College of Medicine (AECOM) had designed more potent PNP inhibitors by retaining the heterocyclic ring system of BCX-34 and BCX-5 and replacing the substituent on the 9-position of the heterocyclic ring with various positively-charged, nitrogen-containing side chains that formed strong contacts in the sugar-binding site of the enzyme. These compounds seemed to have greatly improved pharmacokinetic properties compared to BCX-34 and BCX-5, so BioCryst entered into a license agreement with AECOM for rights to develop these compounds. Two of these compounds entered advanced stages of clinical development. One of these, BCX-1777 (generic name forodesine), was eventually fully licensed to the UK-based pharmaceutical company Mundipharma for development in oncology. A second PNP inhibitor, BCX-4208 (generic name ulodesine), was licensed for a while to Roche for the treatment of psoriasis, but Roche eventually returned the rights to BioCryst where BioCryst continued development through Phase 2 clinical trials for treatment of gout.

An especially frustrating design program was our multi-year effort to develop clinically useful inhibitors of the viral enzyme, RNA polymerase. More recently BioCryst discovered that another compound in the portfolio of molecules licensed from AECOM is a potent inhibitor against hemorrhagic filoviruses, including Marburg and Ebola. The compound (BCX-4430) is currently under active development by BioCryst for treatment of Marburg and Ebola viral infections, with funding from the NIAID division of the National Institutes of Health. NIAID has awarded BioCryst a contract to develop BCX-4430 through Phase 1 for treatment of Ebola virus diseases. A study of BCX-4430 in nonhuman primates infected with Ebola demonstrated an antiviral effect and showed statistically significant survival benefit. BCX4430 is currently in a Phase 1 study.

Under Babu's supervision, the drug design group had impressive success with the development of inhibitors of influenza neuraminidase and serine proteases. The PNP and neuraminidase projects proved to be wonderful learning experiences for guiding future design work, since both enzymes crystallized with packing schemes that permitted ready access to their active sites by diffusion of compounds through the solvent channels in preformed crystals. Consequently, it was possible to perform iterative design of potent inhibitors of these two targets by modeling potential compounds using the native structure, binding the compounds directly to the active site by diffusion into native enzyme crystals, determining the structure of the complex, and seeing directly what additional changes to the inhibitor might be likely to further enhance binding. The PNP project ended up determining the crystal structures of approximately forty complexes that were examined through this iterative process and yielded a wealth of information about factors that would be useful in future design projects. This approach of iterative design also proved to be helpful in making structural changes to improve the clinical potential of potent inhibitors that had undesirable properties, such as toxicity, low solubility, poor bioavailability, poor pharmacokinetics or metabolic instability. By seeing directly what parts of an inhibitor might be modified, without altering the binding interactions, it was often possible to work around problems that prevented a good inhibitor from being a suitable drug candidate.



Ribbon drawing of the PNP trimer, showing BCX-34 bound in the active site.

Following this iterative approach, Babu's team developed peramivir, a potent inhibitor of influenza neuraminidase. Johnson and Johnson (J&J) advanced peramivir up through early Phase 3 US and international clinical trials before deciding that low oral bioavailability of the compound was unsuitable for their commercialization goals. The clinical studies had demonstrated a good safety profile for peramivir, and later in vitro tests against new emerging strains of influenza demonstrated that the compound has activity against multiple strains of influenza, including avian strains that have been of increasing concern as possible pandemic threats. Shionogi successfully completed clinical trials in Japan, which demonstrated that a single intravenous infusion of peramivir is effective for treating seasonal influenza. The intravenous drug is now on the market in Japan, under the trade name of Rapiacta. Peramivir is also approved in South Korea, and licensed to Green Cross Pharmaceuticals, under the trade name

Peramiflu. Meanwhile, BioCryst conducted additional clinical trials with intravenous peramivir (trade name Rapivab) through HHS/BARDA funding. In December 2014 the FDA approved Rapivab (peramivir injection) as a single injection treatment of uncomplicated influenza in adults. This was the first new antiviral treatment for influenza approved by the FDA in 15 years. It was also the first BioCryst designed drug to be approved by the FDA for marketing in the US. In addition, the serine protease inhibitor design program at BioCryst produced a potent inhibitor of the enzyme kallekrein. This orally administered compound (BCX-4161) completed a successful Phase 2 trial for treatment of patients with hereditary angioedema, and is currently in a larger Phase 2 trial treating patients with this devastating disease.

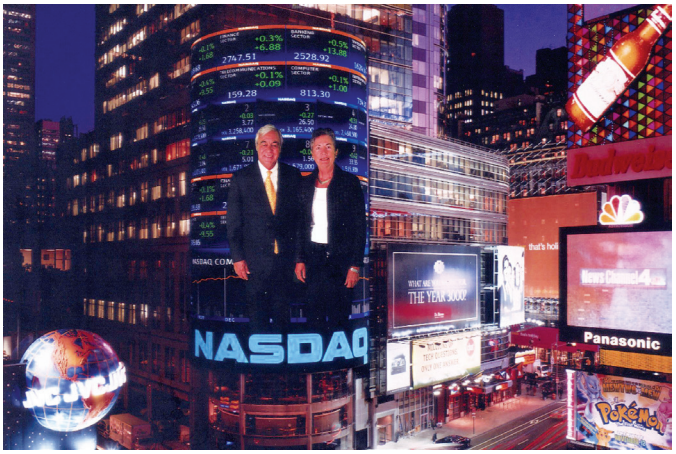
In 2007 I retired as CEO of BioCryst. The company had reached the stage where the focus needed to be on final approval of our drug candidates and commercialization of these drugs. We had established a BioCryst division in 2006 at the Research Triangle in North Carolina to oversee our clinical development and regulatory (i.e., FDA related) activities. The headquarters for BioCryst were moved to North Carolina, after the company recruited Jon Stonehouse to replace me as CEO of BioCryst. All of the research functions have remained in Birmingham under the leadership of Babu who is doing a superb job continuing the structure-based design program.

So what have I learned through these years in the biotechnology industry? First and foremost, it is incredibly difficult and expensive to develop a drug, and the risks involved in moving a compound successfully through the development process are immense. The FDA typically approves 20-30 new drugs each year, although they have done a little better than that recently. A very recent analysis from Tufts University concluded that the average cost of developing a drug currently exceeds $2 billion. What is the chance of a given compound making it successfully through the development process? I have seen figures ranging from 1/500 to 1/10,000. Our experience at BioCryst indicates that those odds are improved by systematic use of structural data during the design and drug optimization process, but a number of initially promising compounds still fail during the clinical stage of development. How long does it take to get a drug from discovery to patients? We started BioCryst in 1986, building initially on several years of research already completed at UAB and SRI, so our experience certainly suggests that it can take many years to get drugs successfully through the development process. The BioCryst drug development programs have required extensive funding over the years, but we have still spent considerably less than the average cost involved in getting drugs to market. Maybe that is attributable to the added efficiency of structure-based design, but we will have to wait and see when the BioCryst compounds now in development reach the market. Above all else, it is clear to me that structure-based design allows a small, focused team to undertake pharmaceutical design and development projects that have generally been the sole purview of large pharmaceutical companies.

The economics of a drug discovery and development company like BioCryst are interesting and somewhat unique. BioCryst has operated in the red, meaning without profits, ever since our founding in 1986. This is not completely surprising considering the long time generally required to move a drug successfully from design, through clinical development and through FDA approval processes. Despite this, BioCryst has remained solvent ever since completing our IPO in 1994. Many of the development costs of the drug candidates have been funded by pharmaceutical partners, and BioCryst has also benefitted from substantial government contracts for developing peramivir and BCX-4430. The deficit between the revenues obtained from these sources and the research and development expenses has been filled over the years by multiple equity offerings. The ability to raise this capital in the equity markets is highly dependent on BioCryst's status as a publicly traded company, which was the original carrot that lured me from academia to pursue the dream of using crystallography to develop important drugs that might eventually make a big difference in the lives of patients.

Before I actually retired as CEO, I was invited to open trading (ring the opening bell) at the NASDAQ stock exchange in recognition of BioCryst's twenty-year anniversary. Bebe and several colleagues from the company, including my long-time Administrative Assistant, Penny Mann, joined me. The main highlight was the picture of Bebe and me together, which was shown off and on during the day on the 100-foot Jumbotron screen at Times Square. I have a blown-up copy of this picture framed in my bathroom at home to remind me each morning of the many exciting, fun and stimulating paths crystallography has allowed me to follow and enjoy during my career.

Charlie Bugg



Bebe and Charlie featured on the NASDAQ Jumbotron in Times Square, in celebration of BioCryst's twentieth anniversary.

Editor: Watch for an extended version of Charlie's memoir that will be available in future on the ACA History Portal. Also take a look at the recent additions to the "ACA Beginnings" section of the website. See: ***www.amercrystalassn.org/history_home.***