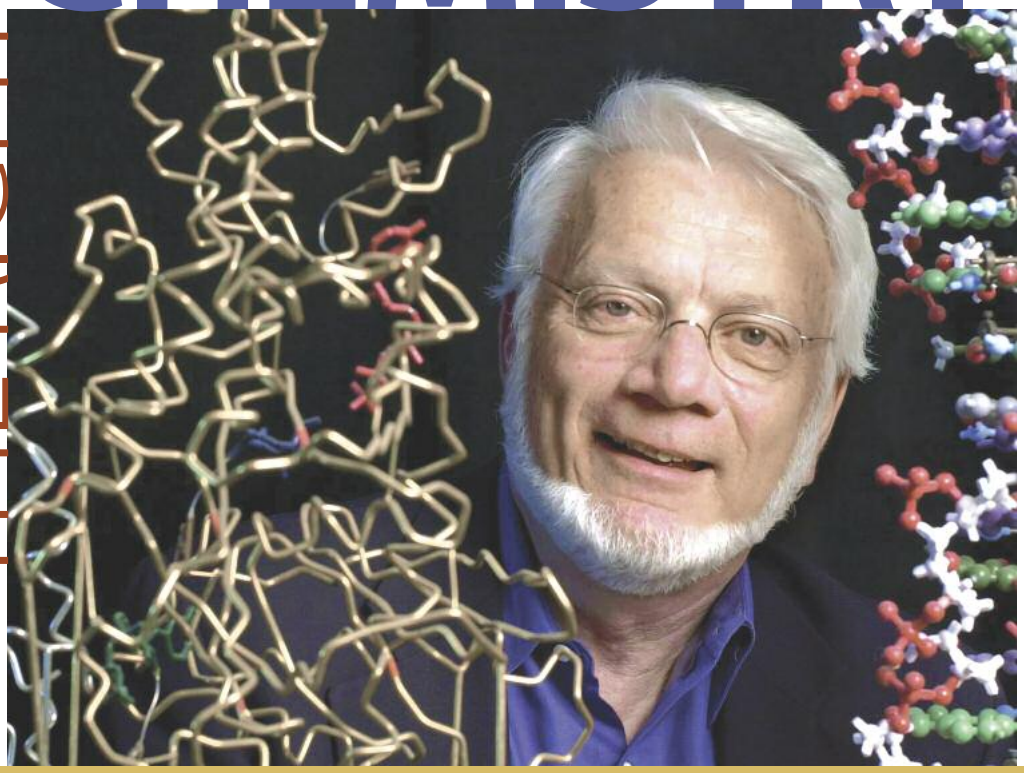
BY KIM RIBBINK

THE RIGHT CHEMISTRY



Thomas A. Steitz, Ph.D., is advancing the discovery of new antibiotics.

Nobel Prize winner Dr. Thomas Steitz has not only made a key contribution to understanding the function of life's protein-making factory, but in co-founding Rib-X he also has played a significant role in advancing the discovery of new antibiotics that have the potential to save lives.

Brilliance begets brilliance, and from early in his career, Thomas Steitz, Ph.D., has learned from and kept company with some of the most highly lauded scientific minds of the 20th and 21st centuries. Over the years, breakthrough discoveries regarding the ribosome have led to Dr. Steitz being often cited and honored by his peers as well as many respected organizations. This year, he joined an elite group of the world's most acclaimed thinkers with the 2009 Nobel Prize for chemistry.

But fame has certainly not gone to Dr. Steitz's head. In fact, if anything, the brilliant chemist — who won the Nobel Prize along with Venkatraman Ramakrishnan, Ph.D., and Ada Yonath, Ph.D., for their work describing the structure and function of the ribosome, the protein-making factory key to the function of all life — is somewhat incredulous at the attention the honor has garnered.

"It's very exciting, of course," he says. "It's had a greater impact on people than I ever would have imagined."

Dr. Steitz, a Howard Hughes Medical Institute investigator, shares the $1.4 million award with Dr. Ramakrishnan of the MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, and Dr. Yonath, Weizmann Institute of Science, Rehovot, Israel. All three used a technology called X-ray crystallography to map the position for each and every one of the hundreds of thousands of atoms that make up the ribosome.

In reality, it is the impact of his discovery that is most significant since it provides a very powerful and profound understanding of life itself as well as having exciting implications at a drug-discovery level. Ribosomes are major targets for antibiotics and it is the potential to further explore drug-discovery opportunities that sparked Dr. Steitz's interest in founding a company, which ultimately became Rib-X Pharmaceuticals.

What excites Dr. Steitz is the implication of the research upon Rib-X's efforts and the potential for a new generation of antibiotic drug discovery.

But he remains first and foremost a researcher and his relationship with the company is one of an advisor and potentially a provider of new ideas.

"It has never been my goal nor will it be my goal to go into applied research as a primary function, but I realize that once a researcher uncovers something new it often can have a practical use, and if medicinal chemistry can do that, that's fabulous," he says.

AN INSPIRING JOURNEY

The journey that took Dr. Steitz to the pinnacle of scientific recognition began under the auspices of his chemistry professor at Lawrence College in Appleton, Wis. Professor Bob Rosenburg, now an adjunct professor of chemistry at Northwestern University, was the young chemist's first inspiration.

"I'd never heard of bonding orbitals and how this could affect the spectra of compounds, but Professor Rosenburg got me excited about the possibilities," Dr. Steitz says.

Dr. Steitz addresses his colleagues at Yale upon hearing the news of receiving the Nobel Prize in chemistry.

Over the years, breakthrough discoveries regarding the ribosome have led to Dr. Steitz being often cited and honored by his peers as well as many respected organizations. This year, he joined an elite group of the world's most acclaimed thinkers with the **2009 Nobel Prize for chemistry**.

The next stop for Dr. Steitz was at Harvard, where he earned his Ph.D. In addition, his time in Cambridge, Mass., allowed him to connect with the first of several Nobel Prize winners along his own journey to Stockholm, including Max Perutz, Ph.D., who won the award in 1962 along with John Kendrew, Ph.D., for determining the structure of the first proteins.

"Dr. Perutz gave a lecture in a huge auditorium with a giant screen over his head on which was projected a slide showing the atomic structure myoglobin, which popped into three dimensions; the whole audience, including myself, went 'oh,'" Dr. Steitz says. "It was the first time any of us had seen an atomic structure in 3-D; this is when I decided this could be the way to understand how molecules work."

The next leg of Dr. Steitz's journey was the result of a discussion that happened during a tennis match, which put him in touch with William Lipscomb, Ph.D., who had begun working on protein structures. Dr. Steitz worked in Dr. Lipscomb's lab on the structure of carboxypeptidase A, one of the key steps he took on the path to determining protein structures. Ultimately, Dr. Lipscomb joined the ranks of the Nobel winners, being named in 1976 for his work in developing a bonding model for boron hydrides based on the results of X-ray structural analysis.

On the recommendation of a fellow researcher, Dr. Steitz took the next big step by joining the lab of protein crystallography pioneer David Blow, Ph.D., at Cambridge University, United Kingdom.

"I wrote a letter to Dr. Blow to ask if I could work in his laboratory and he wrote back to say, sure," Dr. Steitz reminisces.

The time Dr. Steitz spent in Cambridge, U.K., from 1967 to 1970, exposed him to many great scientific minds and ideas.

"This was the major lab in molecular biology in the world; everyone interacted with everyone," he says. "There were seven or more people on the staff who had either won the Nobel Prize or would receive the Nobel Prize in subsequent years."

Among these scientific leaders was Francis Crick, Ph.D., who with James Watson, Ph.D., discovered the structure of the DNA molecule.

"It was my interactions with Francis Crick, as well as Jim Watson at Harvard, and others that stimulated my interest in studying gene expression," Dr. Steitz notes.

In 1970, Dr. Steitz returned to the United States and was named assistant professor of molecular biophysics and biochemistry at Yale University, where he is currently the Sterling professor of molecular biophysics and biochemistry, professor of chemistry and investigator in the Howard Hughes Medical Institute (HHMI).

Over the last three decades, he has been working on the structural basis of gene expression emanating from Dr. Crick's research, referred to as Crick's Central Dogma, which in general describes the normal flow of biological information: DNA can be copied to DNA (DNA replication), DNA information can be copied into mRNA, (transcription), and proteins can be synthesized using the information in mRNA as a template (translation).

"I had been working on many different steps in that process and then by 1995 or thereabouts my colleagues and I had studied almost all of the steps at some level, and while there's still plenty to do, it seemed like it was time to look at the ribosome," he says.

CRYSTAL CLEAR

The ribosome is a huge RNA and protein machine, consisting of 100,000 atoms, that translates RNA sequences into amino acid sequences. The ribosome is composed of two subunits, small and large.

Dr. Steitz notes that since the ribosome is extraordinarily abundant in all cells, making material was not really the issue, but deciding which ribosome to use was.

Scientific discovery is an incremental process and scientists are interdependent by necessity. Dr. Steitz says Dr. Yonath's work was important in that she generated the first crystals of the ribosome around 1980. While the crystals didn't diffract very well, this work showed it could be done, Dr. Steitz says.

In 1995, Dr. Steitz began to pull together the team that would lead to his breakthroughs on the ribosome. This team included postdoctoral student Nenad Ban, Ph.D., and Dr. Steitz's longtime friend and colleague Peter Moore, Ph.D.

By 2000, the team had determined the atomic structure of the ribosome's large subunit, the 50S; that same year Dr. Ramakrishnan and Dr. Yonath separately published structures of the small subunit, the 30S. Dr. Steitz used the Haloarcula marismortui, an archae bacterium living between bacteria and eukaryotes, to map the structure of the 50S. Halo is recognized as a source for good crystals of the ribosome.

All the Nobel winners used X-ray crystallography to map the position of the hundreds and thousands of atoms that make up the ribosome. Through this method, the beam of an X-ray strikes the protein-RNA crystals, leaving a diffraction pattern that can be used to determine the structure of the molecules. (To read more about the process Dr. Steitz used, please see the digital issue.)

The ribosome is the major target of antibiotics — around half of antibiotics — Dr. Steitz says. Antibiotics work by binding to the RNA site of bacterial ribosomes and inhibiting the protein synthesis of bacteria, without affecting the patient. Over time bacteria mutate and become resistant to existing antibiotics, and Dr. Steitz and his group observed structural alterations where antibiotics bind to the ribosome with different sensitivity as a result of mutation. This discovery enabled the researchers to determine why the mutation has the effect it does.

Given these observations, Dr. Steitz saw the potential for developing new types of antibiotics; the question was how best to transfer that knowledge.

Earlier experiences with a couple of pharmaceutical companies related to HIV reverse transcriptase and later a DNA-complex antibiotic carrier had been less than fruitful, and Dr.

Nobel Prize Winner Dr. Thomas Steitz

THOMAS A. STEITZ, PH.D., Sterling Professor of Molecular Biophysics and Biochemistry and Professor of Chemistry at Yale University, is one of three winners of the 2009 Nobel Prize in Chemistry for his work describing the structure and function of the ribosome, the protein making factory key to the function of all life. Dr. Steitz, a Howard Hughes Medical Institute investigator, shares the $1.4 million award with Venkatraman Ramakrishnan, Ph.D., of the MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, and Ada E. Yonath, Ph.D., Weizmann Institute of Science, Rehovot, Israel. All three used a technology called X-ray crystallography to map the position for each and every one of the hundreds of thousands of atoms that make up the ribosome. While the work began as a quest to answer basic questions about the makeup of ribosomes, knowledge of its structure has created targets for a new generation of antibiotics.

"Tom Steitz's relentless pursuit to solve a puzzle at the very foundation of biology inspires us, not only by its intellectual rigor, but also by its potential for the treatment of infectious diseases," said Yale President Richard C. Levin during the announcement on Oct. 7, 2009. "His work is a compelling example of how a quest to answer fundamental questions about life processes can lead to dramatic benefits for mankind."

The instruction manual for the creation of proteins is DNA, but the ribosome is the machine that translates the encoded information to turn it into proteins. Dr. Steitz's work has elucidated the structure and function of the ribosome, an enormously complex ensemble of numerous protein and RNA components.

Dr. Steitz and colleagues built upon research of the past half century to describe in minute detail the architecture of the protein-making machinery. Scientific interest on the ribosome has focused on two major subunits. The smaller 30S subunit binds to messenger RNA that harbors the blueprint for protein synthesis. The second subunit 50S carries out the protein synthesis reaction by adding specific amino acid residues onto a growing protein backbone.

(Above) Dr. Thomas Steitz and his colleagues Sterling Professor of Chemistry Professor of Molecular Biophysics and Biochemistry Member of Yale faculty Peter Moore, Ph.D., and Rib-X CEO Susan Froshauer, Ph.D.

(Right) Dr. Thomas Steitz and the "Ribosome Team" at Yale University, who were involved in the ribosome project that led to the award.

Photo credit: Michael Marsland, Yale Photographer

Steitz wanted to find another way to make use of his ribosomal discoveries.

The idea to form a company began to germinate when Dr. Steitz and John Abelson, Ph.D., met for a pub lunch in Cambridge, U.K. Dr. Abelson was with the California Institute of Technology at the time.

Dr. Abelson co-founded the Agouron Institute, a nonprofit research foundation, in 1978 and participated in the creation of Agouron Pharmaceuticals, a small company that ultimately developed the HIV protease inhibitor Viracept.

"I asked John if it would be reasonable to start a company to make antibiotics and to help me get started," Dr. Steitz says. "During the next year, we tapped some other colleagues at Yale and formed the company."

The company's co-founders included Dr. Steitz, his Yale colleague Dr. Moore, Harry Noller, Ph.D., Robert Louis Sinsheimer, Ph.D., professor of molecular biology at the University of California, Santa Cruz, and another Yale colleague William Jorgensen, Ph.D., whose computational methods are pivotal to Rib-X since they allow the company to interpret the chemistry of the ribosome to design new molecules.

Dr. Steitz approached Susan Froshauer, Ph.D., whom he had known for many years, to head the company.

The founders managed to secure some angel funding, hired a team, established a lab, and began operating in 2001.

Rib-X has exclusive license to the high-resolution crystal structure of the bacterial ribosome revealed by Dr. Steitz and his colleagues.

The company's research strategy combines computational analysis, X-ray crystallography, medicinal chemistry, microbiology, and biochemistry, enabling the company's researchers to rapidly synthesize new agents designed to avoid typical antibiotic resistance mechanisms.

ADVISORY ROLE

Though Rib-X's founder, Dr. Steitz maintains a purely advisory role at the company and continues his research work at the HHMI and as professor of molecular biophysics and biochemistry at Yale.

"While I'm not involved in the research at Rib-X, I'm continuing to work on the whole central dogma issues, including ribosomes, and occasionally we come up with results that are not only valuable and interesting to us but have an impact on what Rib-X might do, so we do continue to contribute," he says.

In fact, HHMI does not allow scientists to work collaboratively with companies so once Dr. Steitz has had research published, he works with Yale and HHMI to determine if it is possible to transfer the information to Rib-X.

"If we do find something useful to Rib-X, then it's in everyone's interest to ensure that we can secure an agreement," he says.

More recently, Dr. Steitz and colleagues determined the structure of the complete 70S ribosome (subunits 30S and 50S associate to form the complete 70S ribosome) and its significance in relation to tuberculosis. It turns out that known TB agents bind to the interface between the two subunits.

"We're in the process of having that paper published and I'm going through the process with Yale and HHMI to see how we can transfer that knowledge to Rib-X," he says.

For Rib-X a potential TB program offers a great deal of excitement, though the company also recognizes that it does not have infinite resources and must therefore ensure its existing programs are fully developed.

The issue of funding is one that concerns Dr. Steitz in the current environment. While biotech companies were quickly able to go public in the late 1990s, the economic slowdown has made things harder for even companies that have demonstrated great clinical success, as Rib-X has done.

"Rib-X has completed Phase II clinical trials with two compounds and has been hugely successful at meeting its goals, yet compared with times past the funding situation is very challenging," he says.

Advancing research remains the cornerstone of what Dr. Steitz and his colleagues seek to do. And an important aspect of research is collaborating with others and sharing one's findings. This takes Dr. Steitz on the lecture circuit in the United States and abroad, as well as engaging in studies with other academic institutions.

He says it's vital to have communication in science and while journals are the primary way of sharing findings, meetings greatly facilitate the process.

"Meetings make concepts much more understandable, but it's also the discussions in the hallways or over a beer that stimulates ideas," he says.

Beyond the lab, Dr. Steitz's leisure pursuits might be described as a combination of geology, botany, and chemistry but at a much more relaxed level. He is a avid hiker (though not rock climber), gardener, and cook.

Dr. Steitz says, "Cooking is, after all, chemistry, putting things together."

His wife, Joan Steitz, Ph.D., is a renowned scientist in her own right and is best known for discovering and defining the function of small nuclear ribonucleoproteins (snRNPs), which occur only in higher cells and organisms. ✦

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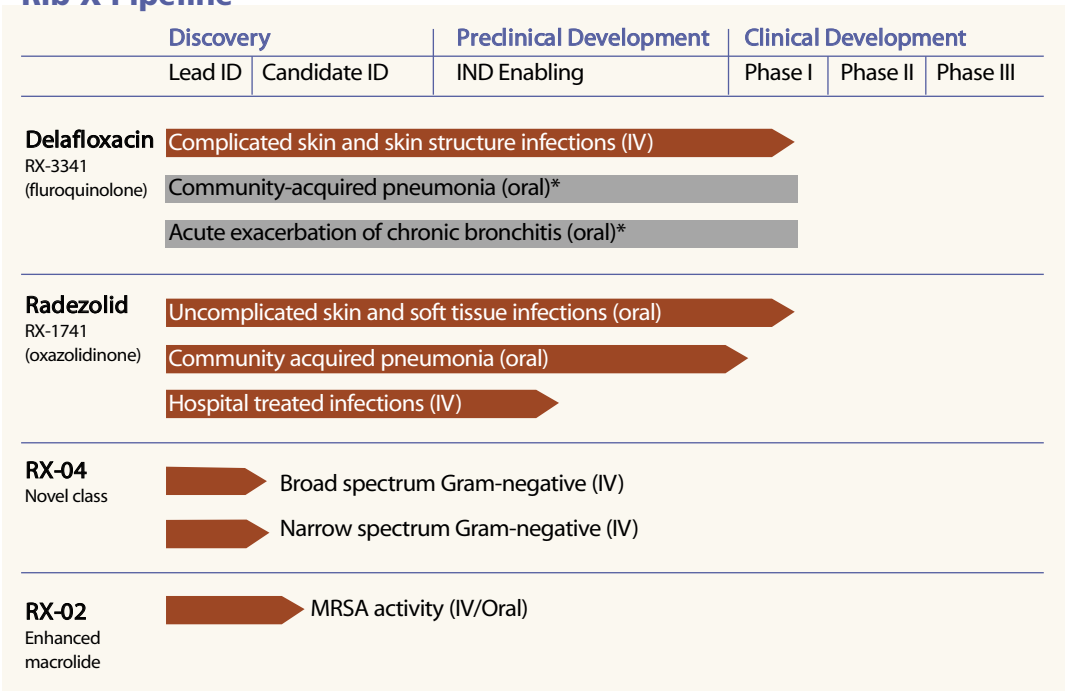
Rib-X Pipeline



* Pre-licensing clinical trial
Source: Rib-X, rib-x.com/pipeline.

A Breakthrough PHASE

Photo credit: Michael Marsland, Yale Photographer

BY KIM RIBBINK

Scientific advances in one field depend on the breakthroughs made elsewhere, and for Thomas Steitz, Ph.D., and others furthering the understanding of the ribosome, several advances over the last four or five decades have been significant to their work.

"One of the things that made a very large difference in the whole field of structural biology is cloning, which happened in the over expression of protein in the late 1970s and early 1980s," he says. "We saw the structure of the first overexpressed protein, DNA polymerase 1 in 1985, and that development absolutely transformed structural biology because it enabled scientists to look at things that are normally rare."

Another key change, he says, is the rate of data collection that can be achieved.

"Synchrotron radiation is much more intense by many, many orders of magnitude and along with the detectors and the computational power that's available, this has led to a significant improvement in the rate of collection in the lab," he says, noting all these were invaluable in overcoming some of the challenges in X-ray crystallography.

The work on the ribosome involved a select field of scientists, starting with Ada Yonath, Ph.D., then Dr. Steitz and Venkatraman Ramakrishnan, Ph.D., then a bit later Harry Noller, Ph.D.

A major stumbling block had been what is known as the phase problem, which is the name given to the loss of information concerning the phase that can occur when measuring the diffraction intensities. Specifically in X-ray crystallography the phase problem has to be solved to determine a structure from diffraction data.

Max Perutz, Ph.D., received the Nobel Prize in 1962 for proving that X-ray crystallography and heavy atoms could reveal the structures of complex, biologically important proteins. Dr. Perutz came up with the structure of hemoglobin while his colleague, John Kendrew, Ph.D., determined the structure of myoglobin, which carries oxygen to the muscles.

For the ribosome, which is 100 times bigger than myoglobins, this method had short comings in the sense that the heavy atoms are not necessarily 100 times bigger.

"I equate this with trying to determine the weight of a ship captain with and without the ship," Dr. Steitz explains. "Theoretically, it's possible to weigh the ship plus the captain, then just the ship and subtract. If it's a row boat it wouldn't be necessary to have a very accurate scale to get the weight of the ship captain, but if the ship captain is on the Queen Mary this is challenge. We now have our heavy atom on the Queen Mary, so how do we get a signal that we can measure?"

The way Dr. Steitz and his colleagues got the signal was to use a heavy atom cluster compound containing 18 tungsten atoms bonded close together.

"If one looks at a very low scattering angle it scatters essentially like one atom, and its scatter is the square of the number of electrons, so the scatter is 18 square times larger than one constant atom," he says. "This gave us a big enough signal so we could basically use the approach Max Perutz used for the hemoglobin and John Kendrew used for myoglobin, except we had to make a bigger signal."

The discovery enabled Dr. Steitz and his colleagues at Yale to publish the complete atomic structure of the large ribosomal subunit in 2000.

X MARKS THE RIBOSOME SPOT

When Dr. Steitz began to consider how to take his breakthrough in mapping the ribosome into a drug development setting he gave careful consideration as to how to achieve the greatest results.

Previous experiences in working with big pharma companies had taught him that companies would not give this type of research their undivided attention. So he decided to found a company that would be focused solely on the ribosome and discovering and developing antibiotics from that science.

Dr. Steitz chose as Rib-X's CEO and President Susan Froshauer, Ph.D., who had done her postdoctoral work in the cell biology department at Yale where he teaches. They had also worked together on a project at Pfizer, using the reverse transcriptase structure from HIV to design new anti-AIDS molecules, where Dr. Froshauer had been

A Research Journey: The Ribosome

1980: Ada Yonath, Ph.D., Institute of Science, in Rehovot, Israel, generates the first three-dimensional crystals of a ribosome's large subunit

2000: Thomas Steitz, Ph.D., and Peter Moore, Ph.D., Yale, solve first structure of the large subunit

2000: Yonath and Venkatraman Ramakrishnan, Ph.D., MRC Laboratory of Molecular Biology, in Cambridge, U.K., separately obtain the first structures of the small subunit

2001: Harry Noller, Ph.D., University of California, Santa Cruz, Jamie Cate, Ph.D., UC Berkeley, and Marat Yusupov, Ph.D., the University of Strasbourg, France, obtain first structure of the entire ribosome

2001: Steitz, Moore, Jorgensen, and Noller co-found Rib-X Pharmaceuticals

2009: Steitz, Yonath, and Ramakrishnan awarded Nobel Prize in Chemistry

bringing external scientists from academia and biotech together to work collaboratively within the company.

"We all realized that the ribosome offered so much chemistry and information that to be successful in activating that science for the purpose of antibiotic drug discovery it needed to be exploited as the focus of a company, not something that would be used peripherally," Dr. Froshauer says. "At Rib-X, studying the ribosome structure is what we do; all of our drug discovery is guided by understanding the three-dimensional chemistry of the ribosome, how antibiotics bind to the ribosome, and what new binding opportunities are that result in inhibition of protein synthesis."

The founders recognized from the start that given the scientific foundation the company had, it could potentially deliver many new classes of antibiotics active against multi-drug-resistant bacteria for years to come.

"We believe we can ultimately become the antibiotics company; many companies that are pursuing antibiotics have just one opportunity or just one class of drugs," Dr. Froshauer says.

Rib-X is building a strong pipeline. The first clinical candidate emerging from the ribosomal chemistry is radezolid, which is the next generation of Zyvox (linezolid), Pfizer's antibiotic, and is in the oxazolidinone class.

Zyvox is a frontline drug to treat serious methicillin-resistant Staphylococcus aureus (MRSA) infections but resistance has been emerging. The importance of the drug has meant many companies have pursued oxazolidinones, not always successfully. Rib-X, with its unique science, was very quickly able to identify a compound to put in the clinic.

Radezolid has successfully completed two Phase II clinical trials in oral form: one for community acquired pneumonia and the second for uncomplicated skin and skin structure infections. The company, which is also looking to do clinical trials with the IV form, anticipates filing an NDA with the FDA around 2013.

The product furthest along, delafloxacin, doesn't come from Rib-X science but was licensed in early on by Rib-X as a complement to radezolid from a business perspective.

"We saw an opportunity to enhance our portfolio and now we have a situation where we would potentially have two products ready to launch at the same time," Dr. Froshauer says. "This would allow us to amortize the salesforce over two products."

The most exciting project in the Rib-X pipeline, Dr. Froshauer says, is its Rx-04 program, which targets multidrug-resistant Gram negative bacteria, such as Escherichia coli and pseudomonas, which is a serious multidrug-resistant pathogen.

"All the drugs coming down most companies' pipelines have activity against MSRA and other largely Gram positive bacteria; there are no drugs that work well against multi-drug-resistant Gram negatives," she says.

In addition, Rib-X employs computational tools to create models that help researchers understand what makes a molecule have Gram negative activity.

"The penultimate goal of the company is to do de novo design of novel agents as the medical need arises," Dr. Froshauer says. ✦

PharmaVOICE welcomes comments about this article. E-mail us at feedback@pharmavoice.com.

Academic Greatness

DR. THOMAS STEITZ — RESUME

2001 – PRESENT. Co-founder and Chair of the Scientific Advisory Board, Rib-X Pharmaceuticals
1986 – PRESENT. Investigator, The Howard Hughes Medical Institute, Yale University
1979 – PRESENT. Professor of Molecular Biophysics and Biochemistry (MB&B), Yale University
2000 – 2003. Chairman, Department of Molecular Biophysics and Biochemistry, Yale University
1984 – 1985. Fairchild Scholar: California Institute of Technology
1981. Acting Director, Division of Biological Sciences, Yale University
1976 – 1977. Macy Fellow: Max-Planck-Institute for Biophysical Chemistry (Prof. K. Weber), Goettingen, Germany: MRC Laboratory of Molecular Biology (Dr. A. Klug), Cambridge, U.K.
1974 – 1979. Associate Professor, MB&B, Yale University
1970 – 1974. Assistant Professor, MB&B, Yale University

HONORS

2009. Nobel Prize in Chemistry
2007. Gairdner International Award
2006. Keio Medical Science Prize
2004. Frank H. Westheimer Medal, Harvard University
2003. Fellow, American Academy of Microbiology
2002. Lawrence University Lucia R. Briggs Distinguished Achievement Award
2001 – PRESENT. Sterling Professor of Molecular Biophysics & Biochemistry
2001. Rosenstiel Award for Distinguished Work in Basic Medical Sciences
2001. AAAS Newcomb Cleveland Prize (awarded February 2002)
1994-2001. Eugene Higgins Professor of Molecular Biophysics & Biochemistry
1991. Member, Connecticut Academy of Science and Engineering
1990. Member, American Academy of Arts and Sciences
1990. Member, National Academy of Sciences
1981. Honorary D.Sc. Degree, Lawrence University
1980. American Chemical Society Pfizer Award in Enzyme Chemistry

ADVISORY POSITIONS

2001 – PRESENT. Scientific Advisory Council, The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia
2001 – PRESENT. Co-founder and Chairman of the Scientific Advisory Board of Rib-X Pharmaceuticals Inc.
1998 – 2006. Board of Scientific Advisors, The Jane Coffin Childs Memorial Fund for Medical Research
1997 – 1998. Selection Committee, General Motors Cancer Research Foundation, Alfred P. Sloan Jr.
1997. NIH/NIAID Reviewer, Innovation Grant Program for Approaches in HIV Vaccine Research
1993 – 2000. Scientific Advisory Board, Skirball Institute in Biomolecular Medicine, New York University Medical Center
1987 – 1992. Committee on Microgravity Research, Space Studies Board, National Research Council