

Lefkowitz is a clinician who still makes hospital rounds. After more than 30 years in the laboratory, he now hopes to help improve the treatment options for heart patients.

Photo: Scott Dingman

OUT OF THE BRONX

Pursuing Receptors and Heart Disease in North Carolina

By Karyn Hede

Bob Lefkowitz doesn't simply walk into a room, he bounces in. On one sunny summer day, he arrives in his office carrying giant yellow and green rubber bands and accompanied by a postdoctoral fellow. "We're doing a little PT [physical therapy] here," he announces, as he ties one end of a band to a table leg and inserts a sneakered foot into the loop at the other end. "Here's what I've been doing," he says to the postdoc, and proceeds to swivel his foot to the right and then to the left. She responds with a few pointers.

"When you've been running as long as I have," he offers, "it starts to catch up with you."

At 57, Lefkowitz is an energetic man, lean and angular. He carries a mere 158 pounds on his six-foot frame. But appearances deceive, and you would never guess by looking at him that what he's been running from is coronary heart disease. His father died at age 63, following his fourth heart attack, and his mother had a heart attack at age 59. It's a disease he knows intimately.

"I knew I had it coming from both sides," he says, but he just pushed it to the back of his mind. Too many other things to think about—and Lefkowitz is not your ordinary heart patient. He can tell you in minute detail exactly what's going on when the heart starts to labor against arteries filling with fat-laden plaque. He helped define what it means, with molecular precision, when the heart starts to fail. He laid the basis for understanding the intimate details, the biochemical drama that silently weakens and then saps the life of 400,000 people a year in the United States alone.

But when his own number came up, well, the mind plays tricks on even the nimblest of brains. "I developed angina," he said. "But interestingly, I could only bring it on by running."

Didn't he, a trained cardiologist and consummate biochemist, realize what was happening? "You know, and you don't know," he responds with his distinctive Bronx accent, still quite recognizable after 27 years in North Carolina as an HHMI investigator at Duke University Medical Center. It was only after nine months of denial that he could ignore the symptoms no longer and, in June 1994, had a quadruple bypass operation.

Since then, he has maintained a strict vegetarian diet, continues to run—despite nagging injuries—and now, from all appearances, leads a charmed life. He is the man who essentially defined receptor biology through his work with adrenergic receptors, the molecules that translate hormonal signals into cellular responses in the heart and other organs throughout the body. Lefkowitz is largely responsible for decoding all of the α and β receptors. He was the first to isolate the β_2 adrenergic receptor (β_2 AR) in 1982; the first to clone the

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α_2 receptor in 1986, the α_1 receptor and all the α_1 and α_2 receptor subtypes (there are nine known adrenergic receptor subtypes); the first to identify and clone the G protein-coupled receptor kinase: adrenergic receptor kinase (ARK) or GRK2, the major regulator of α_1 AR activity; and the list goes on. A member of the National Academy of Sciences, Lefkowitz's office walls are papered with plaques and certificates attesting to his achievements. (See "[Mentor and Data Junkie](#)")

"One of the things that I think has categorized my research throughout the years, and something which I have delighted in, is how hard it is to categorize," he says. "I've had fellows in my lab from every [medical] discipline."

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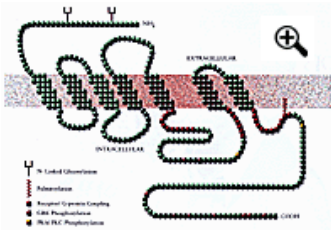
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Road to Research



The schematic representation shows some of the salient features (indicated in color) of a typical G protein-coupled receptor.

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Lefkowitz himself is fundamentally a biochemist and molecular biologist who never intended to make research his career. As a child, he read medical fiction (and detective stories), and it was in the third grade that he decided to become a physician. "Doctors were my heroes," he said. Thus, when the time came, he went to medical school at Columbia University, finishing first in his class. He got hooked on research, however, during a short stint at the National Institutes of Health—and never looked back.

When he started working on adrenergic receptors in the early 1970s, even Raymond Ahlquist, who coined the terms "alpha" and "beta" receptors, believed them illusory. Experiments suggested that they should exist, but scientists had seen only their shadows, the marks they made on the cell. No one had ever proven them real.

Ever the pragmatist, Lefkowitz was undeterred by the idea that receptors may not exist as separate entities. Logically, he was convinced that they existed, so he set out to isolate them.

"Especially when you are young, you have chutzpah... the brazen gall of youth," he says. "I didn't appreciate that it was a risk, and you know what, it never entered my mind that I wouldn't succeed; incredible isn't it? ... I had no reputation to lose."

But he soon gained a reputation, and it wasn't the one he had hoped for. The adrenergic receptor whose identification he reported in a journal article turned out to be another adrenaline binding protein. The scientific press pilloried him.

"I watched his response through this period," said Lewis T. (Rusty) Williams, Lefkowitz's first graduate student and now chief scientific officer at Chiron Corp. "Here he was, a young faculty member trying to make his mark. He wasn't demoralized by the criticism. He just wanted to get the right answer. He probably doesn't even realize that watching his response to adversity made a huge impression on me. I watched him doggedly pursue this goal even through all the criticism. He kept going until he got it."

It took a couple of more years, but he found the receptor. Since then, Lefkowitz and his army of graduate students, medical students and residents, and postdoctoral fellows (some 160 since the mid-1970s) have methodically dissected and reassembled the essential elements of a signaling system that has been a model for receptor biologists.

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From Worms to People

It is hard to overestimate the importance of G protein-coupled receptors (GPCRs) and their cognate G proteins. They seem to be everywhere in the human body, regulating heart rate; basic endocrine function; the senses of sight, smell and taste; pain tolerance and even the high of cocaine. And that's just for starters. Sequence homology data suggest that there are hundreds, perhaps thousands, of GPCRs still to be identified in the human genome. Five percent of the genome of the worm *C. elegans*, one of the first model organisms to be sequenced entirely, for example, consists of genes for GPCRs, says Henrik Dohlman, a former Lefkowitz protégé who is now an associate professor of pharmacology at the Yale University School of Medicine.

It might seem that in view of how long GPCRs have been studied and the many scientists now working in the field, there would be no more room for innovation. But nothing could be further from the truth, says Lefkowitz. Even after all these years, the field continues to produce many surprises.

Three years ago, a genetic variation was discovered that protects some people from developing AIDS despite repeated exposure to HIV. The variation turned out to be a slight modification of a molecule called CC-chemokine receptor 5 (CCR5), the GPCR that HIV uses to enter a T cell, which is its preferred entry point into the immune system. In separate work, Dan R. Littman, an HHMI investigator at New York University Medical Center, and Ed Berger at the National Institutes of Health showed that CCR5 is a co-receptor for HIV. Suddenly the world of HIV research came knocking on Bob Lefkowitz's door, and he found himself speaking before international AIDS conferences.

As with AIDS, so with epilepsy. Lee Limbird, Lefkowitz's first postdoc, is exploring a weak "agonist" or activator of the 2 subtype receptor to control that disease. She and her colleagues at Vanderbilt University are starting a clinical study to see if clonidine, a blood pressure medication, can reduce the frequency of epileptic seizures. Other research in her lab suggests that weak partial GPCR agonists can lower blood pressure without sedative side effects. "The clinical applications seem unlimited," she says. Right now, fully half of all prescription medications work through the GPCR cascade, she points out.

And just this year, HHMI investigators Charles Zuker at the University of California, San Diego, and Linda Buck at Harvard Medical School, working separately, identified the first human bitter taste receptors. No surprise: These, too, are GPCRs, as are a series of smell receptors that Buck and her colleagues previously identified. It is the way Buck found the smell receptors that illustrates the power of Lefkowitz's work. Her group launched its search of the genome under the assumption that the receptors would look like cousins to the GPCR family that Lefkowitz defined early in his career.

"I would say that over the last decade or so, Lefkowitz laid the groundwork that now allows us to expand the study of these receptors in many systems," says Buck—and there are literally thousands of varieties of GPCRs in the sensory systems alone.

In fact, the first GPCR to be identified and cloned, rhodopsin, translates light signals into images in the brain. It was Lefkowitz and his group who discovered in 1986 that ARs

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are strikingly similar to rhodopsin, exhibiting what has come to be called a "seven transmembrane structure"—a portion of the protein that snakes its way through the membrane seven times. When he further predicted that GPCRs would turn out to be a superfamily of receptors, he made quite a splash.

But that was then, and Lefkowitz has moved on. He is, after all, a clinician who still makes hospital rounds and, after 30-plus years of lab work, is ready to make a foray into the clinical arena.

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Into the Clinic

In the early 1990s, with the help of Duke surgical fellow Carmelo Milano, postdoctoral fellow Walter Koch and a team of other trainees, Lefkowitz began to examine the possibility of using the β_1 AR system as a kind of molecular ventricular-assist device—a way to help a failing heart increase its ability to pump. Their idea has its origins in Lefkowitz's early work and in basic knowledge of how the heart responds to stress.

Lefkowitz knew that when the heart is under stress, it increases the production and release of the stress hormone norepinephrine, which binds to β_1 ARs located on heart cells. This stimulation initially helps the heart beat faster and more powerfully, but it quickly becomes self-defeating: The norepinephrine-stimulated receptors become desensitized by a second molecule called β_1 -adrenergic receptor kinase (β_1 ARK).

The group demonstrated in 1994 that mice genetically altered to produce excess β_1 ARs have supercharged hearts that beat faster and stronger than normal mice. They reported in the April 24, 1994, issue of *Science* that these mice mimicked normal animals treated with the human heart failure drug dobutamine—even though the mice didn't receive any drugs.

This finding was quickly followed in 1995 with a report on genetically altered mice that produce too much β_1 ARK in their hearts. These mice mimic aspects of congestive heart failure in humans. When the researchers injected a synthetic adrenaline-like hormone, the mice couldn't contract their heart muscles as well or increase their heart rate as much as normal mice. The transgenic mice displayed a lack of hormone response similar to that seen in patients with heart failure. The study confirmed what *in vitro* studies suggested: Too much β_1 ARK desensitizes the β_1 AR system so that the heart can no longer recognize hormone stimulation.

Once they were convinced that increased levels of β_1 ARK reduce the ability of the heart to respond to hormones, the researchers wondered whether they could restore heart function by blocking β_1 ARK. As it happened, Koch had been offered a position in the surgery department at Duke and saw it as an ideal opportunity to take Lefkowitz's work into the clinic, with the goal of delivering genes, or perhaps small molecules, to treat or even prevent heart failure.

Koch designed a molecule that competes with the normal β_1 ARK in heart cells, thereby diluting its effect. First in transgenic mice and now in rabbit models, Koch, Lefkowitz and their surgical colleagues have shown that the β_1 ARK inhibitor keeps heart cells sensitive to hormone stimulation when normal heart cells would have become desensitized.

Their work has caught the interest of several pharmaceutical companies that are now testing their own β_1 ARK inhibitors. Although neither Koch nor Lefkowitz will speculate on when clinical trials might begin, it is clear the clinical progress pleases Lefkowitz.

"My father died in 1963, right on the cusp [of research progress]," he says. "The first coronary artery surgery was performed in this country in the mid-1960s. My father never had a cath [coronary artery catheterization], he never had anything. If he were here, I believe he would be pleased,"

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Lefkowitz says.

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Mentor and Data Junkie



The mid-1970s found Bob Lefkowitz playing mentor to postdoc (and future HHMI investigator) Marc Caron.

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Just about everywhere you look in receptor biology—indeed, biomedical research—you find someone who trained with Bob Lefkowitz. They total some 160 so far.

Walter Koch, a former Lefkowitz postdoc and now close collaborator at Duke University Medical Center, once picked up Lefkowitz at the airport in Ohio when Koch was a still a graduate student at the University of Cincinnati. "I had read all his papers," Koch recalls. "He'd been publishing so long, I was expecting somebody 65, and here he was only 45. I was amazed."

Lefkowitz's most enduring contribution to biology may, in fact, be his dedication to mentoring and his unflagging devotion to his students. When asked how he thinks he will be remembered, he replies that he hopes he'll be remembered as someone who made a contribution to science and as "somebody who trained a helluva lot of people."

Talk to his former students and a consensus emerges that Lefkowitz has a gift for helping students succeed. Virtually all of the alumni of his laboratory, including four current or former HHMI investigators, have gone on to establish independent research careers.

"He has succeeded as a mentor because he has made a decision to resist the things that distract too many scientists," says Henrik Dohlman, a Yale pharmacologist and former graduate student in Lefkowitz's lab from 1982 to 1988. "He rarely travels, never chaired a department, doesn't own a biotech company, doesn't edit a journal. His important and noble focus has been on the education of his students. I always tell my students a mentor should do three things: inspire, instruct and inform. He does all these very well."

But there's one thing you'd better have if you want his attention: Data. "Bob is sort of a data junkie," says Rick Cerione, professor of molecular medicine at Cornell University and former Lefkowitz postdoc.

Koch concurs, "He'll talk to you about anything—rock music, movies, whatever—but if you really want his attention, you'd better have some data. He really, truly gets genuinely excited when you have a good result, and the beauty of it is he gets excited every time you show it to him. I've shown him a result in the lab and then presented it at a lab meeting, and he gets excited all over again.... It's contagious. He really motivates you."

Lefkowitz himself has divined the secret for success in science. "I always tell my trainees there are four secrets to success in science: the first is focus; the second is focus; the third is: You get the idea."

He tells the story of a microscope he bought in medical school. It had been passed down through several generations of students and was showing its age. Lefkowitz would be studying his histology slides and he'd look away to his notes. When he looked back, the microscope would be slightly out of focus.

"I learned after a while just the right amount of pressure to put on that fine-tuning knob to hold it in focus, look away, and—when I looked back—it was still in focus. That's what I have to do with the fellows—exert just this little pressure. I

find I go out of town for a week, I don't talk to a fellow, I come back, they're losing their focus! Every experiment suggests an infinite number of possibilities. The magic is to see the path through, to see where there is a clear space. That's what I do."

Lefkowitz makes sure his students not only stay focused but also focus on big questions."Bob has a way of taking complicated situations and reducing them to the simplest forms," says Dohlman. "His attention is always focused on one question ahead of the game. He taught me how to think big. But the most important thing is that he helps people so eventually they won't need him. He has a way of asking questions instead of dictating answers, so that any success I've enjoyed is my own."

Says Lefkowitz, "There is no way to develop a person's independent creativity unless they have some room, both to make mistakes and to develop their own ideas. Nothing gives more confidence than having an idea and showing it's true. In order to let that happen, you have to let go some."

But make no mistake, says Lee Limbird, Lefkowitz's first postdoc and now associate vice chancellor for research at Vanderbilt University Medical Center, he's the kind of guy who you go to with data. What you learn from him very quickly is that data are the currency of research. If you go to a scientific meeting and don't have data, you may as well not be there, she adds.

"The biggest thing Bob taught me was that sometimes your data look like garbage, but if the same result comes up a couple of times the data are telling you something and you'd better listen," says Sheila Collins, a former Lefkowitz trainee and now associate professor of psychology and pharmacology at Duke.

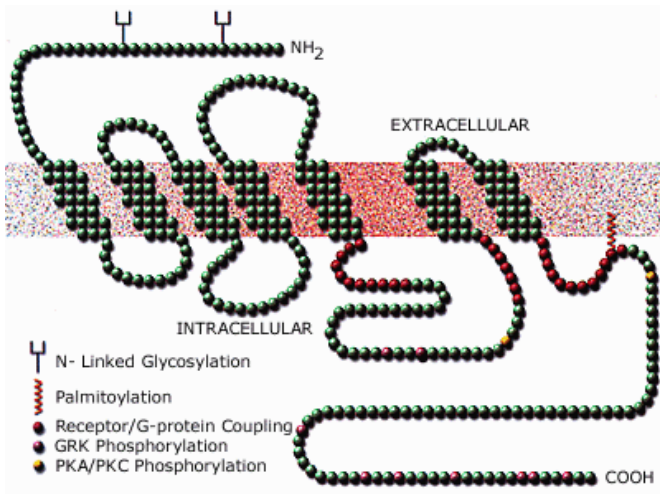
Lefkowitz has had a large lab almost since the beginning. Today he has 30 people working in it, including students, postdoctoral fellows and technical and administrative staff. With that many people, he has to be judicious with his time. Without data, it can take a while before you see him. "If I couldn't get an audience with him because I was data-less, I would sometimes call him from next room to get his advice," Limbird recalls, laughing.

Of course, everyone has an Achilles' heel, and former graduate student Rusty Williams, now at Chiron Corp., says Lefkowitz's is lab work: The guy was not meant for the lab bench.

"Bob took great pride in doing things himself, but he wasn't very good in the lab," Williams says. One day Williams was working in the lab trying to isolate a substance he'd been working on for a week. He had a homogenate of tissue and had spun down the solid, trying to get supernatant, the liquid portion of the sample. "At this point Bob strode into the lab and said 'Here, let me show you how to decant the sup [supernatant],'" says Williams. He then proceeded to dump the liquid right down the drain. "My jaw just dropped," says Williams. "After that I learned to stay clear if he came into the lab."

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The schematic representation shows some of the salient features (indicated in color) of a typical G protein-coupled receptor.

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