



# Endeavor

Summer 2005



## The Virus Issue

AIDS, SARS, and Nanotechnology

# Endeavor

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Summer 2005

Viruses are responsible for some of the major health challenges of our time—SARS, AIDS, and the flu, for example. Paradoxically, viruses may also offer potential new therapies for the treatment of disease. This issue of *Endeavor* features some of the investigators at The Scripps Research Institute whose research focuses on these enigmatic species.

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## Scientists Describe Antibody that Neutralizes Most HIV Strains

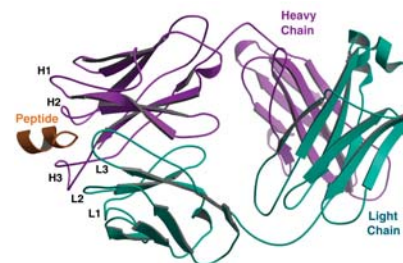
A group of scientists from The Scripps Research Institute and several other institutions has solved the structure of a rare human antibody that broadly neutralizes human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS).

Because neutralizing antibodies attack the virus before it enters cells, they can prevent HIV infection if they are present prior to exposure to the virus. An HIV vaccine would seek to elicit these neutralizing antibodies—just as existing vaccines against diseases such as measles, polio, hepatitis B, and hepatitis A elicit neutralizing antibodies against those viruses.

However, this is easier said than done. The body makes many antibodies against HIV, but they are almost always unable to neutralize the virus. Nonetheless, the immune systems of some patients with HIV have beaten the odds and have produced effective neutralizing antibodies. The structure of one of these, called 4E10, is described in a recent issue of the journal *Immunity*. Significantly, the structure shows what an effective HIV-neutralizing antibody can look like.

“This antibody is very broadly active,” says Scripps Research Professor Dennis Burton, Ph.D., who led the research with Scripps Research Professor Ian Wilson, D.Phil. “It neutralized nearly 100 different viral strains of HIV from all over the world. [During tests in the laboratory], every one of them was neutralized.”

Reference: *Immunity*, 22, 163-173 (2005).



Scientists have solved the structure of this rare human antibody, which may have implications for the development of an AIDS vaccine. (Image by Rosa Cardoso generated with Molscript.)

## Viruses That Make Good Molecules Go Bad

Ask any four people to give you the correct definition of irony, and you are likely to get four different answers, perhaps all equally correct. That's because every field has its own examples of irony—from dramatic irony, where the intended meaning of a line is the opposite of its literal meaning, to political irony, where a bill passed into law accomplishes the exact opposite of what was intended.

Now scientists at Scripps Research are describing a situation that can perhaps be called “immunological irony,” in which an immune system molecule causes immunosuppression.

Scripps Research Professor Michael B. A. Oldstone and his colleagues Bumsuk Hahm, Matt Trifilo, and Elina Zuniga are reporting that certain viruses have the ability to subvert a class of antiviral molecules called type I interferons, an important part of our innate resistance to viruses. Type I interferons normally are produced early on in a viral infection and help the body clear the infection by inhibiting viral replication and by kick-starting other parts of the immune system.

“Interferon has always been considered a protective molecule—a good molecule [produced] to help the host,” says Oldstone. But, he adds, the protective effect of type I interferons depends on the state of the cells’ differentiation in which they are produced.

If type I interferons are produced within the mature form of a kind of immune cell known as the dendritic cell, they interfere with viral replication and are good for the host and bad for the virus. However, if the interferons are produced within a dendritic precursor cell, they actually shut down the expansion and development of mature dendritic cells, which is bad for the host because it suppresses a vital part of the innate and adoptive immune system and thus helps the virus.

Oldstone and his colleagues report that they observed this immunosuppression of dendritic cell precursors *in vitro* and *in vivo* when the cells were infected with two separate viruses—measles and a rodent virus called lymphocytic choriomeningitis virus.

Oldstone has made a career of studying host–virus interactions and his work has been recognized with numerous prizes, including the J. Allyn Taylor International Prize in Medicine and the Biomedical Science Award from the Karolinska Institute. This latest paper presents evidence that viruses can suppress the immune response with type I interferons—which should be suppressing them. The team suggests this may occur because of a previously unrecognized interferon-induced signaling pathway in dendritic cells.

Immunosuppression is a major problem in viral infections because it can make an otherwise not-so-lethal infection lethal. The main cause of death in patients with measles is, in fact, secondary bacterial or parasitic infections. In other words, virus-induced immunosuppression can open the door to infection by an “opportunistic” agent like *Mycobacterium tuberculosis* or *Staphylococcus*. And it is this secondary infection that is often fatal.

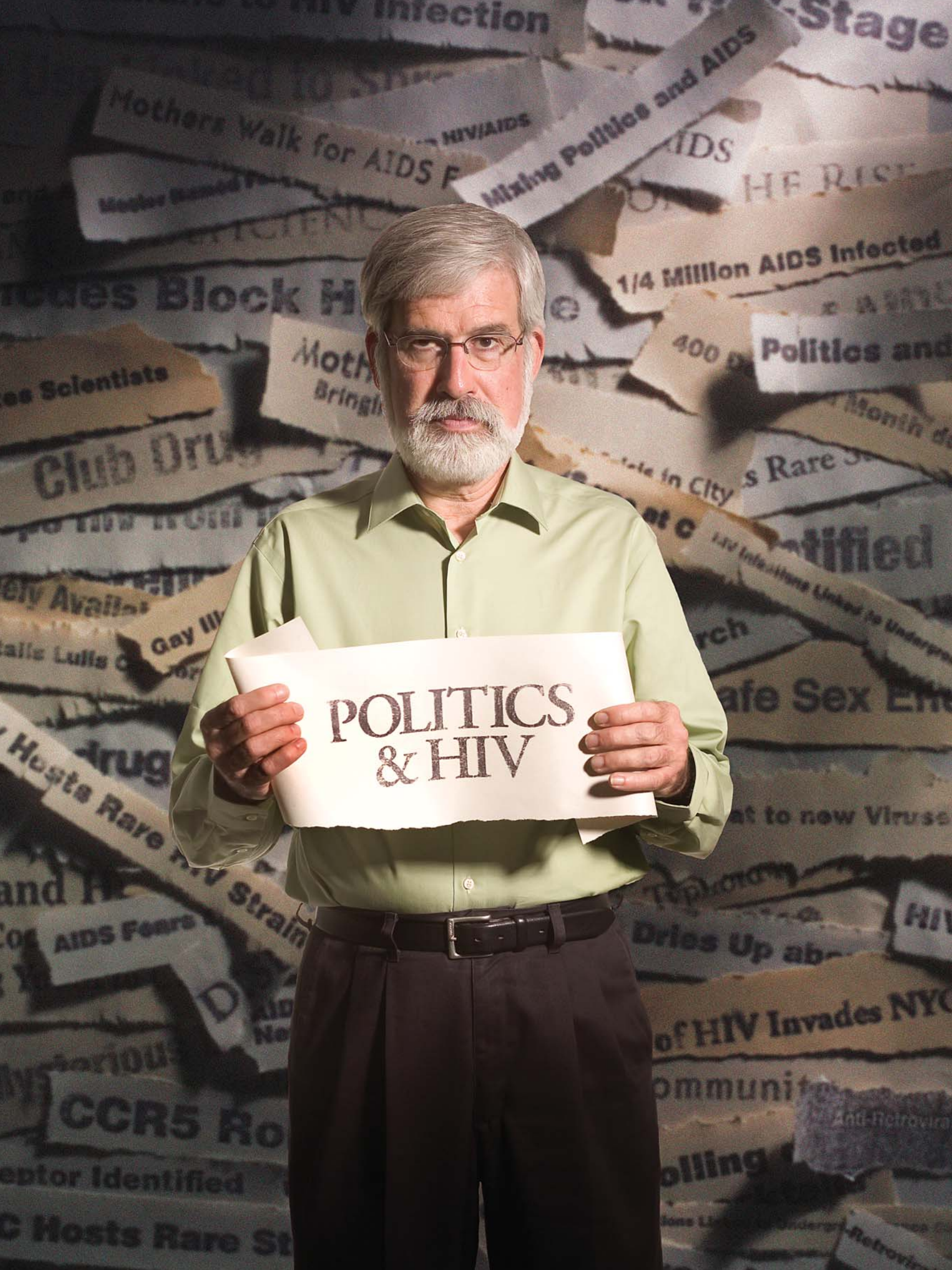
These findings represent a major advance in our understanding of how some viruses interact with their animal hosts. Because this mechanism was shared by two viruses from two different families, says Oldstone, this subversion of interferon’s action is something that may be common to a number of viruses that infect humans. Furthermore, the research suggests a possible new pathway to target for reducing the immunosuppressive effects of these viruses. Indeed, Oldstone points out that the suppression of the immune system caused by measles has been harnessed for medical use in the past. Before steroids were discovered, measles was used to treat a number of terminal autoimmune diseases, such as autoimmune renal disease.

“Since you know what the pathway is that causes this suppression, you could [potentially] make drugs to block that pathway,” says Oldstone.

Reference: *Immunity*, **22**, 247-257 (2005).



Measles, the most contagious infectious agent known to human-kind, has afflicted many societies, as this drawing by a sixteenth-century Aztec shows. (Drawing from the *Códice Florentino*.)



**POLITICS  
& HIV**

# Political Science

## DON MOSIER CONFRONTS THE PROBLEMS OF AIDS RESEARCH

In the world of HIV research, the political is never very far from the scientific. Scripps Research Institute Professor Don Mosier, M.D., Ph.D., has lived with both since he began research on the virus in the mid-1980s. Since that time, he has built a reputation as one of the world's foremost specialists in HIV/AIDS, a title with no hint of exaggeration but not without its own sense of irony.

He's been around long enough to be surprised, even a little amused, at the reaction to the news item that appeared in the media about a new killer strain of HIV/AIDS. In early February, a rare strain of HIV was diagnosed in a New York City man that appeared to lead to a rapid onset of the disease and was resistant to nearly all anti-retroviral drugs. Public health officials immediately called a news conference to announce what one researcher called "a scary phenomenon" and strongly urged those at risk to avoid dangerous behavior.

"I thought the whole incident was a wonderful public health message," Mosier said, "but there wasn't much science behind it. The fact is that drug-resistant viruses are more prevalent today, so it really was a roll of the dice this would happen. But right now you can't call it a new strain."

Still, after so many years of relatively positive news, this discovery brought with it renewed fears about the disease, recalling the early years of the epidemic—a time before today's anti-viral drugs existed, when all the world knew about AIDS was its terrible and unforgiving headlines. (During the 1980s, the number of AIDS cases and deaths of people with AIDS surged dramatically; in the United States, a drop in new cases and mortality only began in 1996, with the introduction of combination anti-viral therapy.)

It was during those early years that Mosier first became acquainted with HIV, an introduction that would eventually lead to his decision to devote his scientific life to making sense of it.

### A COMMITMENT TO RESEARCH

In reality, it was not a surprising choice for Mosier, who was an undergraduate during the 1960s and, like

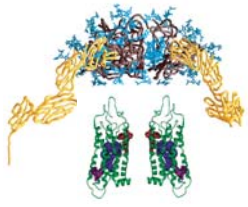
so many others, joined the causes of the day. He grew up in southern Indiana and went to Indiana University. In 1965, he left Indiana and moved on to the University of Chicago, eventually getting both an M.D. and a Ph.D. in immunology. After Chicago, Mosier moved to London for some post-doctorate work with the English public health service, returning to the United States for his internship at Children's Hospital in Boston coupled with a research fellowship at Harvard University.

In Boston, Mosier worked in pediatric pathology at a time when bone marrow transplants were still an experimental treatment. The patients were children from all over the country, kids with genetic diseases who were down to their last chance. Mosier saw the ones who didn't survive.

"As an intern, if you pay attention, you see a lot more problems than you can ever hope to do anything about," Mosier said of his time in Boston. "I saw enough problems as an intern to keep researchers busy for decades. It seemed much more satisfying to work in research and find the tools that would help physicians."

*"I saw enough problems as an intern to keep researchers busy for decades. It seemed much more satisfying to work in research and find tools that would help physicians." —Don Mosier, M.D., Ph.D.*

From then on, Mosier was committed to research. In 1972, he went to the National Institutes of Health and stayed for six years, working in the areas of virology and immunology with people like Anthony Fauci, currently the director of the National Institute of Allergy and Infectious Diseases. Mosier was working on another virus that suppressed the immune system in mice when the HIV/AIDS epidemic was officially recognized. After leaving the NIH, he moved to Philadelphia and the Institute for Cancer Research, teaching immunology at the University of Pennsylvania as well. He moved to La Jolla in 1985 and that's when he first became fully caught up in HIV, the science as well as the politics. →



It wasn't until 1996 that Don Mosier and other researchers gained a major insight into how HIV worked. The virus used two co-receptors to gain entry to the immune cell.

“You have to remember that when HIV appeared in the 1980s, there was virtually no political response at the federal level,” Mosier said. “When I got to California in 1985, I discovered that the state’s reaction to the disease was actually far better than what the federal government was doing. Willie Brown and the people from San Francisco in the state legislature started a research program that was the best funded in the country.”

So, in 1987, he abandoned his viral research in mice and went to work on HIV. It was a pretty straightforward decision: “I realized that my mouse virus wasn’t going to cut it—and we had a real HIV virus to work with. If I wanted to work on HIV, then I had to work on HIV.”

### THE EMERGING EPIDEMIC

Mosier and others in the newly emerging field knew HIV/AIDS was a bad disease. What they could not know or even imagine was how quickly it would spread around the world. In the mid-1980s the disease was working its way through the United States and the developed world, but it had yet to begin its devastating sweep through sub-Saharan Africa and the Far East. Scientists were focused more on finding a treatment for a rare disease than on stopping an epidemic that had not quite happened yet.

“There was a lot of optimism about a vaccine during that time,” Mosier remembered. “It was just around the corner then—and it’s still just around the corner today. Because I was the chairman of the first AIDS review committee at the National Institutes of Health and later of the UC (University of California) AIDS taskforce, I got quickly involved in the politics that surrounded the issue. There’s always a political dimension to a disease as serious as this.”

This was also the time that he’d discovered the usefulness of a genetically bizarre mouse known as the SCID—pronounced skid—mouse. What makes SCID (severe combined immunodeficiency) so unique is that the animal has virtually no immune system. Mosier had first been introduced to this immunological oddity at the Fox Chase Cancer Center outside Philadelphia in 1984. SCID was a natural mutation and the only reason the line survived is because the mutant mice were housed in a germ-free containment facility.

What made SCID mice such a useful tool was the fact that when Mosier transplanted human

immune system cells into SCID, the human cells thrived. SCID mice were an almost perfect living laboratory for viral research.

For Mosier and other early researchers HIV presented a fundamental mystery. At first, they thought it just killed CD4 cells, the white blood cells that play a key role in the immune system. But the course of the disease seemed far more complicated than that single hypothesis could explain. Maybe HIV was simply a mutation of some other virus that already existed—because there were other well recognized primate viruses around. (Mosier also became interested in the Epstein Barr virus, a herpes virus that can cause a number of diseases ranging from mononucleosis to several types of lymphoma.) Each year it became apparent how much more they had to do.

For the first ten years, Mosier said, researchers worked on a type of the HIV virus that could be grown in the laboratory. It just wasn’t the one that was circulating in most of the world’s HIV patients. Mosier began to collect patient virus samples—including a unique sample from a patient just seven days after infection—and injecting them into the SCID mice.

The patient viruses did unexpected things, some were not as pathogenic as he expected, and they behaved differently in various types of cells.

It wasn’t until 1996 that Mosier and other researchers gained a major insight into how HIV worked in patients. The virus used two co-receptors to gain entry to the immune cell—CCR5 and CXCR4. Naturally occurring chemokines, molecules that stimulate movement of immune cells to sites of infection, bind to these receptors and can, to varying degrees, block HIV infection. The CCR5 co-receptor is the primordial receptor, the one used by virtually all viruses after primary transmission of the disease. But HIV can also use CXCR4, although it generally does so in later stages of infection. As a result of that discovery, much of the work done with the laboratory-bred virus—which uses CXCR4—turned out to be less relevant to patients, and virtually useless for vaccine studies.

### A POTENTIAL TARGET

For Mosier, the CCR5 receptor quickly became a potential target for therapeutic intervention, and one with a built-in safety advantage.

“One attractive aspect of CCR5 is that there is a mutation in humans that essentially knocks out the expression of that co-receptor protein,” Mosier said. “This is the reason people get excited by the idea of CCR5 blockers to keep HIV from entering the immune cell. There are thousands of people walking around out there without any of these co-receptors—so blocking it seems relatively safe.”

And because human immune cells thrive in SCID mice, Mosier’s model is closer to humans for the purpose of HIV/AIDS study than to monkeys—who carry the original virus in its simian form. In his mind, this is critical to the creation of a workable vaccine.

Closely related to this is another aspect of Mosier’s research—he calls it an “interesting sideline”—into the origins of HIV resistance.

Some studies have suggested that the CCR5 mutation that confers resistance to the virus goes back centuries to a time before the disease actually existed. A mutation that offered protection against the plague was first suspected but tossed out when Mosier found that mice with the CCR5 gene mutation still got the plague. The next suspect was smallpox but Mosier has pretty much ruled that out as well. When exposed to mousepox, the CCR5-mutated mice were more susceptible to the disease.

Today, Mosier is trying to map out the mutations necessary for HIV to change from the CCR5 co-receptor to the CXCR4. One of the characteristics that makes HIV such a difficult virus to treat is its extraordinarily high mutation rate—100 million different viruses generated in the space of a year. Some of these mutations turn out to be lethal, some turn out to be mistakes, but it only takes five mutations to switch co-receptors from CCR5 to CXCR4, making these mutations a critical aspect of any potential therapy with CCR5 inhibitors.

#### **A SIMPLER SOLUTION**

He has also become interested in a simpler form of protection from HIV that uses the idea of the CCR5 blocker but offers a less complicated approach. “In the last three or four years we’ve been studying the potential of microbicides—treatments that inhibit sexually transmitted diseases. We’ve been looking at a class of modified chemokine molecules called PSC-RANTES that could be used in a

topical microbicide. I’m interested in it because if a vaccine is ten years out, there are lots of other compounds that could be used to prevent HIV infection. PSC—which is a very potent anti-viral—is one of them.”

Unfortunately, using a CCR5 blocking agent like PSC in a gel, along with other microbicides to block various sexually transmitted diseases, is not a commercially attractive target at the moment because, unlike antiviral cocktails, it isn’t a high profit market. However, Mosier says, some new microbicide work is being done through funding by the Bill and Melinda Gates Foundation and the WHO Global AIDS programs.

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*“Today you have 20-year-old people who are getting the disease because the introduction of anti-viral cocktails has lulled us—and them—into a false sense of security.” –Don Mosier, M.D., Ph.D.*

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The most recent media scare notwithstanding, Mosier does believe that the world is losing ground in the fight against HIV/AIDS because other global threats such as SARS and bioterrorism have pushed it aside. He has grown increasingly concerned because of a number of emerging trends including an increase in multi-drug resistance plus a rise in the rate of sexual transmission.

“Today you have 20-year-old people who are getting the disease because the introduction of anti-viral cocktails has lulled us—and them—into a false sense of security,” he said. “In reality, that treatment bought us five years because you don’t live forever on these drugs. As a result, we have a generation of young adults who think they’re immortal and think that anti-virals are curative. They’re not. We’re going to need new drugs.”

Promising new treatments are on the horizon—his work on CCR5 blockers is one area that holds tremendous promise—but these treatments are still years, possibly decades, away. In the meantime, a lot of things are slipping away: “Our sense of urgency is sliding, prevention is sliding, funding is sliding. You can’t get away from the main political issue—we can’t move forward in the medical field, particularly with a disease like HIV/AIDS, without significant federal funding. And you can’t have significant federal funding when so many other things are put in line ahead of it.”

•Eric Sauter





# Launching Therapeutic Drugs Into Inner Space

SCRIPPS RESEARCH TEAM USES VIRUS-BASED NANOPARTICLES TO TARGET TUMORS

Armed with therapeutic drugs, virus-based nanoparticles injected into the body move along the bloodstream toward their target—a cancer cell. The body’s macrophages spot a few of these nanoparticles and gobble them up, but most of these stealthy, infinitesimal specks survive. When the nanoparticles reach the cancer cell, they invade it, releasing a cargo of potent drugs.

This is the scenario envisioned by Marianne Manchester, Ph.D., an associate professor of cell biology at The Scripps Research Institute. Her research, which focuses on using drug-loaded viruses directed to specific sites in the body, is funded by the National Cancer Institute for \$4 million over eight years. This work holds great promise for treating cancers such as colon carcinoma, breast tumors, and some types of brain tumors.

But why use nanoparticles for this mission? Why are these miniscule warriors so effective?

“We need something of an appropriate size that can be introduced into the body, go into the circulation, and bind to the sites we’re interested in reaching,” says Manchester, who came to Scripps Research as a postdoc from the University of North Carolina in 1993. “Basically, the bigger the particle, the more noticeable a foreign body is and the more attention it attracts,” she says. “Nanoparticles can move around undetected for a long time.”

Nanoparticles continue to gain attention and earn respect. They’re so small they are measured in nanometers, billionths of a meter. To put this in perspective, the width of this letter “I” is about a million nanometers. These nanospheres—which, under an electron microscope, look like soccer balls scattered on a playing field—provide new ways to deliver specifically targeted, potentially life-saving drugs.

In this project, Manchester is enlisting the aid of two viruses. “We’ve always tended to think of

viruses as pathogens, as the bad guys, but now we’re re-conceptualizing them as materials, platforms for drug-delivery,” says Manchester. She and her team are currently using two well-characterized icosahedral viruses: a plant virus, cowpea mosaic virus; and an insect virus, called flockhouse virus.

“The goal of this project is to make tools that can be used non-invasively. We hope to revolutionize the way cancer is treated—to get away from biopsies, and slash-and-burn chemotherapy, where you treat the whole body even though the tumor is just in one small location.” She adds that another advantage of this approach is that, because the drugs are directed to one small, specific site, higher doses can be used.

*“We hope to revolutionize the way cancer is treated.”*

—Marianne Manchester, Ph.D.

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## THE TEAM APPROACH TO PROBLEM-SOLVING

Manchester says it was measles that led her into this current project, or, more specifically, her previous research on the measles virus.

“I was studying virus receptor interactions in measles, using some peptides we found that can inhibit attraction between the measles virus and one of its cellular receptors. When [Professor] Jack Johnson came to Scripps in ’95, he had the idea that we might try to use viral nanoparticles to display these types of peptides, to use them as a therapeutic.” Johnson, a molecular biologist, is an expert in x-ray crystallography, and a crucial role player in this project, Manchester says.

“Jack’s contribution, his knowledge of the x-ray structure of the particle, is invaluable. We need that information to figure out how to make the particles go where we want them to.” →

She adds that her team uses a variety of techniques to see where the particles go. “We have movies where you can watch the particles flow through blood vessels. We can see them dock at exactly the sites where we want them to bind. It’s really exciting.” The use of fluorescent tagging makes this particle-watch particularly dramatic. Once they enter the targeted cells, the tagged particles, under a fluorescent microscope, light up like tiny Christmas trees. “This way it’s easy to see where they go,” says Manchester, “and truly fun to watch.”

Other team members include: M.G. Finn, Ph.D., associate professor in the Scripps Research Department of Chemistry who is an expert on the use of viruses as chemical building blocks for catalysts and materials; Anette Schneemann, Ph.D., an associate professor in the Department of Molecular Biology who focuses on the molecular mechanisms of virus assembly and the structure-function relationships of virus particles; Heidi Stuhlmann, Ph.D., an associate professor of cell biology who is a specialist in identifying genes that control vascular system development; and John Lewis, Ph.D., a research associate in her lab.

*“We’ve always tended to think of viruses as pathogens, as the bad guys, but now we’re re-conceptualizing them as materials, platforms for drug-delivery.”*  
—Marianne Manchester, Ph.D.

“It’s absolutely essential that I have people working with me who bring particular skills to the project that I don’t have,” Manchester says.

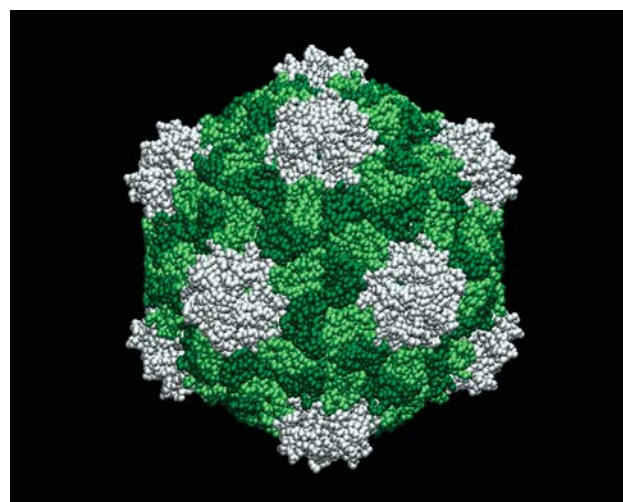
This multidisciplinary pooling of expertise led the team to call their approach the “rational chimera design,” an intriguing name in that it evokes an image of the mythological fire-breathing beast with the head of a lion, the body of a goat, and a serpent’s tale.

“Yeah, that’s us all right,” Manchester laughs. “And if only we could get the fire to blow out the tumor, that would be a truly novel approach.”

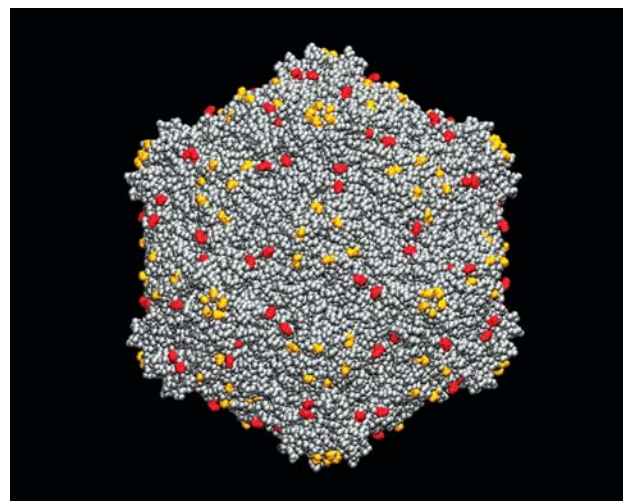
#### WHERE THEY’VE BEEN AND WHERE THEY’RE GOING

The choice of a virus as the basic building block in designing the particles means the researchers have plenty of raw material.

“The virus, when injected into a plant, for instance, grows on its own,” says Manchester.



One of the viruses Marianne Manchester is working with as a vehicle for drug-delivery is the cowpea mosaic virus (CPMV). Like most plant viruses, CPMV is delivered by insects into plant cells, and like most plant viruses, CPMV has little need for its viral envelope to facilitate entry into cells. These envelopes are basically just rigid, stable containers.



An important part of Marianne Manchester’s research is attaching peptides to the virus, and attaching them in the right places so the particle can find the desired target. Here, areas on the Cowpea mosaic virus that are potential attachment sites are shown as colored spheres.

“It contains the genetic information to duplicate itself and make many, many copies.”

Once these copies are harvested, the next step is to attach the right peptides to the virus, and attach them in the right places, so that the particle can find the desired target. This work—Manchester calls it “decorating” the virus—involves, she admits, a lot of trial and error.

So far, the team has designed five different types of particles, all with different characteristics. Perhaps

the trickiest part of making these particles effective has been to orient the attachments so that the particle will find the tissue it's supposed to find. "We want the business end of the particle to be properly decorated with the right proteins in the right locations," she says. "Just as the polarity of a magnet is crucial to its 'binding,' the binding end of the molecule has to be placed in the right direction for it to find its target."

During what the team calls Phase 1 of this project, the researchers learned that some particles were more difficult to work with than others. Some were not stable and fell apart. Others didn't attach to the particle's surface or maintain their ability to interact with tumor cells.

Manchester is pleased with the progress her group has made. "Now, we can successfully target different types of tumors, we have a solid understanding of how to design the particles, and we know how to recognize a good target," Manchester says; her tone is a mixture of confidence and exhilaration. She adds that in coordinating her lab's efforts, she hasn't let her eye stray from the end goal—to one day get these materials into clinical trials to see if they can reduce or eliminate tumors in people.

The team is moving toward that goal. In Phase 2, the researchers will try to maximize the efficiency of targeting and eliminating tumors in a live model. "We'll be dealing with issues of toxicity, side effects, and dosing—the pharmacology of these particles."

When asked what difficulties she expects in Phase 2, Manchester says, "Difficulties?" as if this is absolutely a foreign word to her. "Oh," she laughs, "it's all going to be real smooth sailing, I'm sure." Then, turning back into the thoughtful scientist, she says, "It's a good question, but I'll have to get back to you on that."

#### **WHEN DID YOU KNOW YOU WANTED TO BE A SCIENTIST?**

The question of when she knew she wanted to be a scientist, however, is one she has no trouble fielding.

Back in her freshman year of college at the University of Colorado, Manchester's biology instructor, Larry Gold, put her on the road to a career in science.

"He was so passionate about molecular biology, and his class focused on experimentation, designing

experiments to figure out the answer. I thought it was fantastic."

Another major influence at the University of Colorado was Karla Kierkegaard, Ph.D., now an associate professor of microbiology and immunology at Stanford University who specializes in RNA viruses. "She was another one of my first professors and had just started up her lab, working on poliovirus replication," Manchester recalls. "She had so much energy. She was a model for me: through her example, I realized what a scientist could be like."

In her junior year, Manchester worked in a lab with Marvin Caruthers, an expert in nucleic acid chemistry and biochemistry who still makes his academic home at the University of Colorado. "His lab was huge, full of people from all over the world—Spain, France, Germany, England, Wales, and China," says Manchester. "I liked the mixture of nationalities and approaches." She adds that this multi-flavored experience prepared her well for her work at Scripps Research.

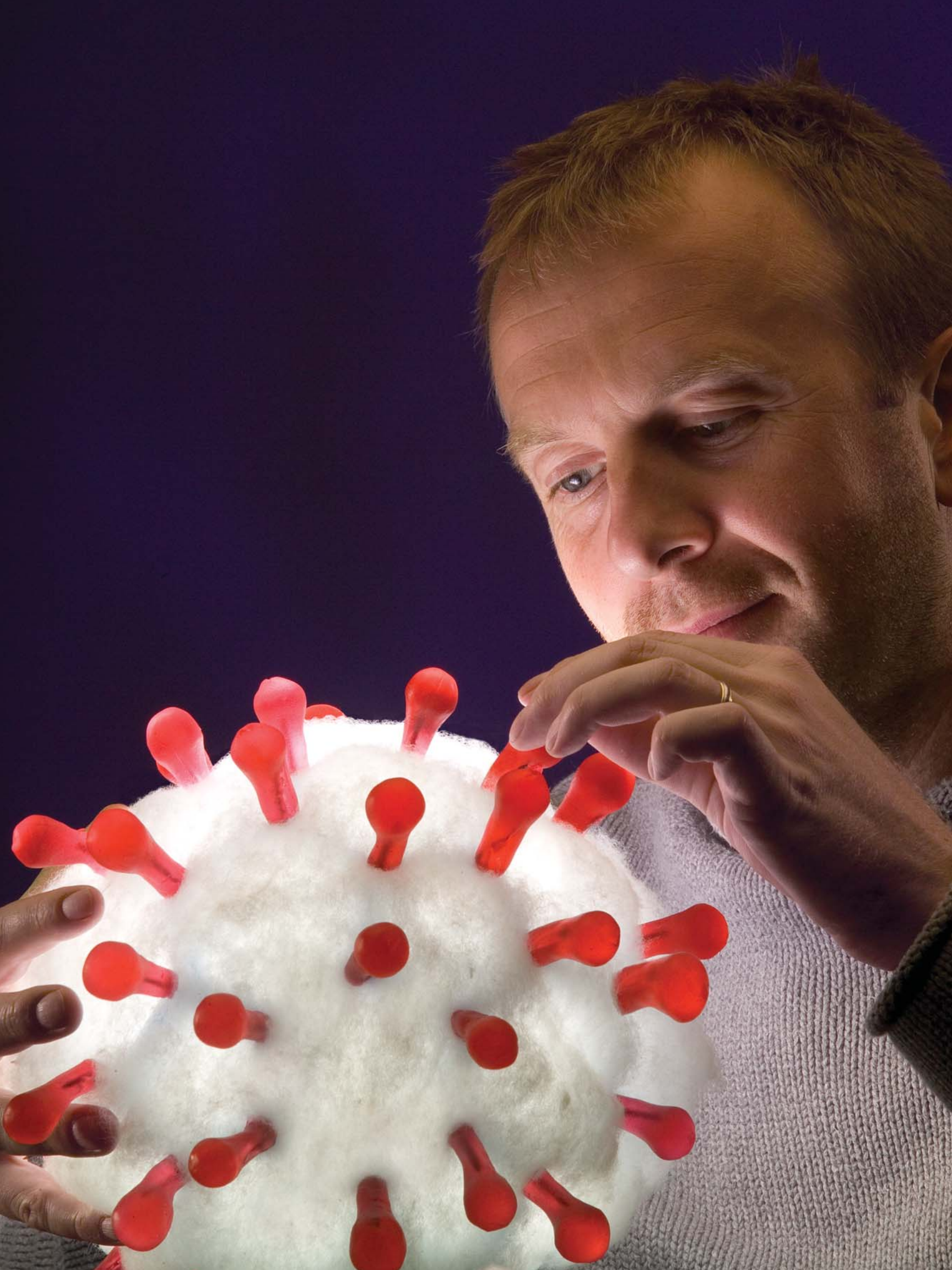
"Not only do we bring a multidisciplinary approach to problems in my lab here, this approach is endemic at Scripps. When we first started on this project, we moved into a new building called the Center for Integrative Molecular Biosciences. This building is full of people with different expertise—chemistry, structural biology, microscopy, and other skills. This is as good as it gets for a scientist. If any of us need another perspective on a problem we're trying to solve, all we have to do is walk down the hall and tap on someone's shoulder."

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*"Not only do we bring a multidisciplinary approach to the problems in my lab here, this approach is endemic at Scripps." —Marianne Manchester, Ph.D.*

Manchester says that science has come a long way from the model of one scientist working alone to solve problems. "So many problems in science now are bigger than what one individual can tackle," says Manchester. "The National Cancer Institute has been wise to promote interdisciplinary programs that bring people together to talk with each other to blend different types of expertise. This approach is going to lead to a quantum leap in cancer treatment."

•Jeff Worley



# Attacking an Epidemic

## A CLOSER LOOK AT SARS

It's 8:35 on a cloudy Monday morning this past winter, and I am walking through the corridors of the Center for Integrative Molecular Biosciences (CIMBio) building at The Scripps Research Institute, to find a meeting held by a group of researchers to discuss aspects of a novel "coronavirus" called SARS-CoV—the virus that causes severe acute respiratory syndrome (SARS). I am already late.

Two years ago, a terrifying new virus was spreading disease from communities as remote as the Guangdong region in Southern China to those as familiar and urban as Hong Kong and Toronto. This virus was spreading fear, too—everywhere. During that spring in 2003, media outlets worldwide were reporting on the disease caused by the virus, which was dubbed SARS. From a few cases, the emerging disease would soon erupt into an epidemic, infecting thousands, killing hundreds, and making dust masks a fashion statement.

Nearly two years to the day after cases of SARS were first reported in the press, I enter a conference room next to the laboratory of Associate Professor Peter Kuhn, Ph.D. Here, a meeting is already underway of a group funded in June 2004 through a major government contract to discover more about SARS-CoV. Kuhn is the contract's principal investigator, and he conducts this meeting twice a month to discuss progress and problems involved in the group's effort.

Two years ago, this group didn't exist—but then neither did SARS. When the disease first appeared in the winter of 2002–2003, the virus SARS-CoV had never before infected humans. Before it was contained by that summer, the virus had quickly spread to more than two dozen countries, including the United States, and had infected 8,098 people and claimed 774 lives.

This morning, Kuhn and his colleagues are talking about everything from solving the structures of SARS-CoV proteins to finding better ways for the 30 or so scientists at Scripps Research and a few other institutions who are involved in the research to share information.

Death from SARS is terrible, which may explain in part much of the public fear surrounding the virus. It infects epithelial cells in the lung and gut and over the course of a week causes high fevers, aches, diarrhea, and dry coughing. The virus also triggers a severe immune response in the upper respiratory tract, leading to pneumonia. In severe cases, the lungs of people with SARS fill with fluids, and many die, literally, by drowning.

At this meeting, I am aware from the different accents that the individuals in this group come from a variety of countries. But as the meeting goes on, I realize they also come from every corner of campus and from nearly every department, with backgrounds in cell biology, chemistry, virology, physics, and computer science. In fact, the main things they have in common this morning are bagels, coffee, and their dedication to understanding SARS.

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*"If we want to come up with new ideas about therapeutic interventions—just ideas, not interventions, we've got to understand how [the virus] works." —Peter Kuhn, Ph.D.*

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### A BROAD, JOINT EFFORT

In a sense, the epidemic was finished before most of the real scientific work on SARS could begin. The 2003 outbreak was contained not through modern biomedical science, but rather through "shoe leather" public health—the good, old-fashioned strategies of monitoring, reporting, and isolating cases.

Stories still occasionally appear in the press related to SARS, but they are more like endnotes. Gone now, in the wake of the successful containment of the disease, are the days when the daily tally of infections and death counts dominated the headlines. Today, the stories about SARS focus on follow-up research.

In the last two years, scientists have been trying to develop methods to respond faster to emerging diseases like SARS at a proteome level where drug discovery can have an impact. Specifically for the SARS case, the team has →

## Screen Yields Small Molecules That Target SARS Virus



Professor Chi-Huey Wong and his team screened some 10,000 compounds for their ability to protect kidney cells infected with the SARS virus.

In the theatre of world health, severe acute respiratory syndrome (SARS) is a four-letter word—and one that entered our lexicon in dramatic fashion in February 2003.

Today, the legacy of our successful effort to contain SARS can be found in the numerous pages devoted to aspects of SARS infections on the Web sites of the World Health Organization and the U.S. Centers for Disease Control and Prevention (CDC). A recent review of these sites revealed detailed guidelines that cover everything from detecting and reporting new cases of SARS to guidance on the proper transport of SARS patients in airplanes.

Yet there was surprisingly little about how to treat SARS in the patients who have it. There are no specific drugs for SARS or for containing the SARS virus.

The CDC recommends that doctors give SARS patients “the same treatment that would be used for a patient with any serious community-acquired atypical pneumonia.” For instance, a doctor might treat a SARS infection by administering ribavirin, an antiviral used to treat hepatitis C and infant pneumonia, and perhaps by administering corticosteroids to the patient as well. But there are no existing drugs available for treating SARS specifically—the way that there are drugs for treating HIV.

Recently, an effort to find other compounds that might be effective against the SARS virus was undertaken by a team of scientists at The Scripps Research Institute in La Jolla, California, and at Academia Sinica in Taiwan.

The effort, reported last year in the journal *Proceedings of the National Academy of Sciences*, was led by Scripps Research Professor Chi-Huey Wong, Ph.D., who holds the Ernest W. Hahn Professor and Chair in Chemistry and is a member of The Skaggs Institute for Chemical Biology at Scripps Research. The work is highlighted in the September 2004 issue of *Nature Reviews: Microbiology*.

Wong and his colleagues used a high-throughput screen in which they infected kidney cells in culture

*continued on page 14*

been trying to understand the virus and to find new ways of treating SARS-CoV infections. Currently, there are no specific drugs for treating SARS as there are for treating AIDS, for instance. And there is no vaccine.

Why worry about SARS now that it has disappeared? The concern is that if SARS emerged once, it or a similar epidemic will emerge again. Also, last summer, the National Institute of Allergy and Infectious Diseases (NIAID), one of the National Institutes of Health, added SARS-CoV to a list of emerging diseases that have potential for use as a bioterror weapon. It's considered a category C agent—less dangerous than some other pathogens, but still dangerous enough to warrant concern and a concerted effort to study the virus.

Because the SARS epidemic was contained before large numbers of people were exposed to the virus, there is no widespread immunity in the general population.

“Given the fact that the epidemic was controlled so rapidly there was no opportunity to develop such ‘herd’ immunity,” says Scripps Research Professor Michael Buchmeier, Ph.D. “As a population, we are no more protected against SARS than we were in the first outbreak.”

So far, the complete DNA sequence of the SARS coronavirus has been solved, and a number of laboratories have found possible therapeutic leads. One team led by a Scripps Research investigator found about 50 compounds out of a library of 10,000 that offered cells some protective effect against the SARS virus (see sidebar). Another group recently reported that a common antidepressant inhibits the virus. Other efforts have yielded high-resolution three-dimensional structures of some of the virus's main proteins. But there is still much to understand.

“If we want to come up with new ideas about therapeutic interventions—just ideas, not interventions,” says Kuhn, “we've got to understand how [the virus] works.”

One way to go about this is to look at the virus's proteome, which is what Kuhn's group is doing, funded by a \$14.5 million contract from the NIAID. The contract, titled “Functional and Structural Proteomics of SARS Coronavirus Related Proteins,” is one of seven large contracts funded by the agency to apply proteomics, the

study of proteins, to various pathogens. The overall goal of the contract, as stated on the NIAID's web site, is to find new targets for the next generation of diagnostics, therapeutics and vaccines.



Before it was contained in the summer of 2003, the SARS-CoV virus had spread to more than two dozen countries, had infected 8,098 people, and claimed 774 lives.

The Kuhn group, which includes scientists at Scripps Research, the nearby Burnham Institute, and the Bay Area's Palo Alto Research Center, has set out to catalog the different proteins that SARS-CoV makes, determine what role these proteins play in the viral life cycle, and figure out how these proteins interact with human proteins within the cells that SARS-CoV infects.

"The idea was really to apply a state-of-the-art molecular approach to attacking these diseases," says Buchmeier, who is one of the investigators on the contract. The strength of the project is that it combines the efforts of a number of different laboratories with complementary skills in structural and functional biology. Applying their wide expertise, these researchers will be working together to solve the three-dimensional structures of proteins and to determine what they do.

SARS-CoV encodes about 28 proteins, says Kuhn. "[But] we don't know much about them," he says. "Some of them we know their function, some of them we have a putative function, but lots of them we don't know anything."

As a complement to the NIAID-funded project and cross-project collaboration at Scripps Research,

several of the SARS proteins are membrane proteins that will benefit from the technology developed as a part of a \$12.5 million grant titled the "Joint Center for Innovative Membrane Protein Technologies" funded last year by the National Institute for General Medical Science (NIGMS). Three of the leaders on the NIGMS grant are also lead investigators on the SARS CoV proteome project—Kuhn and Scripps Research Professors Kurt Wüthrich, Ph.D., and Raymond Stevens, Ph.D.

#### PROBLEMS WITH THE SARS PROTEOME

As a starting point, Kuhn and his colleagues have been trying to produce the various SARS-CoV proteins that help the viruses infect, replicate, assemble new virus particles, and escape to infect more cells.

This is not always a simple matter. About two-thirds of the SARS-CoV genome is one large piece of RNA (termed by biologists an "open reading frame") that encodes two enormous polyproteins called ORF-1A and ORF-1B. These in turn contain about 16 different protein domains that play a role in viral replication.

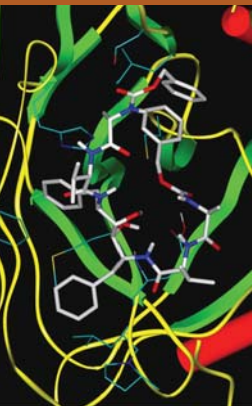
The problem is that nobody knows the exact boundaries between the proteins. "How they all fit together, we don't know yet," says Kuhn.

The situation is analogous to trying to read a sentence where all the words are jammed together with no spaces: Only if you know where each space should be is it easy to read. (Only if you know where each space should be is it easy to read.) In fact, the situation is even worse, because rather than being a sentence of a few dozen letters, the ORF protein is a sentence with about twenty-one thousand letters and includes overlapping words.

*"All viral proteins have been difficult for structural studies, and SARS is no exception." –Ian Wilson, D.Phil.*

Adam Godzik, Ph.D., of the Burnham Institute is applying what is known as bioinformatics to sort this out. "The pre-analysis is very important," says Godzik. "We're trying to make educated guesses as to which pieces are different proteins and what are the functional pieces."

Even when the putative proteins are identified on paper, another hurdle is simply getting those →



New modeling of SARS-CoV can help scientists understand its binding mechanisms.

with SARS and screened some 10,000 compounds for their ability to protect the cells from dying.

Included in these 10,000 compounds were a few hundred drugs that have already been approved by the U.S. Food and Drug Administration for treating other diseases, ginseng and about 1,000 other traditional Chinese herbs, several hundred chemicals that inhibit a class of enzymes known as proteases (the SARS virus has its own protease), and about 8,000 synthetic compounds, including aminoglycoside and oligosaccharide compounds. Wong assembled the library using a technique he invented called programmable one-pot synthesis—a technique Wong uses to quickly assemble many types of carbohydrate structures.

Out of this library of 10,000 compounds, the scientists found about 50 that at reasonable concentrations offered the cells some protective effect against the SARS virus.

Several of these 50 were compounds that are either FDA-approved drugs in use to treat other conditions or are commonly used herbal supplements. And a few more are in the process of clinical development.

The protection exists in many of these cases because the compounds interfere with some part of the virus's lifecycle—such as the entry of the virus into a new cell or the assembly of new virus particles within an infected cell.

For instance, the SARS virus requires its own protease enzyme in order to assemble new virus particles, and Wong took that into account when he designed the 10,000-compound library, adding several protease inhibitors to the mix.

One of the compounds that most effectively inhibited the SARS virus was a protease inhibitor called TL3, which Wong described a few years ago with his Scripps Research colleagues John Elder, Art Olson, Bruce Torbett, and others. TL3 is an interesting molecule because it has the ability to effectively inhibit the proteases made by both human and cat immunodeficiency viruses. It surprised us, says Wong, that TL3 can also inhibit the SARS protease with  $K_i$  in the nanomolar range, even though the protease from SARS is quite different from HIV and FIV.

•Jason Socrates Bardi

proteins expressed so that they can be solved. This is no small task. Scientists often have to find an exact cocktail of salts, buffers, and solutions from among virtually limitless possibilities to persuade the cells to make the protein of interest.

“All viral proteins have been difficult for structural studies, and SARS is no exception,” says Scripps Research Professor Ian Wilson, D.Phil.

“Currently, there are four international SARS-CoV structural genomics efforts in France, China, Japan, Taiwan, [and we] communicate closely with the French and Taiwanese groups,” adds Scripps Research Professor Raymond Stevens, Ph.D., one of the lead investigators of the SARS project. “With the exception of the intact virus and recognition motifs, all of the groups are having great difficulties expressing soluble, stable, and functional proteins from the SARS-CoV proteome.”

One solution they are applying, Wilson adds, has been to identify the functional domains of the individual proteins within the ORF to increase chances of success. Even if the whole proteins don't crystallize, some of their domains might. So far, the scientists have been successful in expressing and purifying 10 unique protein domains.

Wüthrich, who won the 2002 Nobel Prize in Chemistry, and his group are using nuclear magnetic resonance (NMR), the technology behind hospital MRIs, to screen proteins from SARS-CoV to see which might be prepared biochemically in a way that enables their structures to be solved. The NMR can tell them if the proteins aggregate, for instance, or behave in ways that might prevent them from yielding their secrets to NMR, crystallography, or both.

Then the teams tackle the structure using one of these two technologies or a third, called cryo-electron microscopy, that Scripps Research Professor Ron Milligan, Ph.D., is applying to the problem. Electron microscopy can often provide structural information on an intact virus, but in this case, the technique is limited because particles of SARS-CoV are not uniform in size and shape. Without this uniformity, it's impossible to get high-resolution images, says Milligan. “You can, however, get some information on the envelope proteins—the proteins that are sticking out of the surface,” he adds, “and you can get a bunch of pretty pictures.”



## OFF TO A GOOD START

“We are making rapid progress—there is no question,” says Buchmeier. “We already have structures submitted for publication.”

Recently, Wüthrich and his colleagues managed to solve the structure of a small 83-amino acid protein called nsP7 (an acronym that stands for “non-structural protein number seven”) in a matter of a few short months. Given that it can sometimes still take years to accomplish all of these steps, getting the structure in just a few months was an amazing feat.

“It’s quite exciting,” says Wüthrich.

In addition to trying to solve SARS protein structures, the scientists are also looking at how SARS-CoV proteins interact with other proteins and with potential drug candidates. For this line of research, the project makes use of an innovative new instrument developed at the Palo Alto Research Center (PARC), an independent research company that is a wholly owned subsidiary of Xerox and famous for inventing many of the tools of the modern computer, such as the mouse and the graphical user interface.

A few years ago, a collaborative entity known as the Scripps-PARC Institute for Advanced Biomedical Sciences was created to apply innovative engineering technologies to grand biomedical challenges. Kuhn, the head of Scripps-PARC in La Jolla, recalls how at the beginning of the collaboration a group from both institutions sat around a table and decided that one of the biggest challenges was coming up with a generic way of testing the interactions a protein might have with many other proteins or chemicals.

In response, PARC scientists developed an innovative technology called the enthalpy array, a thermodynamic instrument that detects interactions between two proteins or a protein and an inhibitor by measuring the tiny amount of heat released when they bind to each other.

As part of the SARS contract, Richard Bruce, Ph.D., head of Scripps-PARC in Palo Alto and an adjunct professor at Scripps Research, uses the enthalpy array to look for interactions between SARS-CoV proteins and to screen for potential inhibitors of viral enzymes. Significantly, the enthalpy array works with sample sizes significantly less than might be required with traditional instruments of this type—normally one mL or more. The enthalpy array works with drops 1,000 times smaller.



Scientific information flow is a key element for the success of a complex research program such as the functional and structural proteomics of SARS. Microsoft provided the collaborative tools of SharePoint Portal Server and Live Meeting that support the daily data handling and sharing amongst the participants of the project.

This is a major advantage, says Bruce. “You don’t need to have that much material to get an answer.”

## PRIORITIZE, PLAN, EXECUTE

As an outsider in the meeting I’m attending, I quickly become lost when the discussion turns to the actual proteins, referred to by what sounds like code names: E11, F2, 9B, 7B, and others.

The discussion goes back and forth across the table from one scientist to another discussing one protein or another. This one has a good score in the screen, but it has too many cysteines. Will this one express? Will this one fold? That one expresses insolubly, and the other one probably would express insolubly if it expresses at all. But is it functionally important? This other one looks good enough to express.

“Let’s prioritize, plan and execute,” says Kuhn, and they do. Several people begin to write action items on the board: tasks to be executed, new ideas to be discussed, and strategies to be tested before the next meeting.

Shortly thereafter, the meeting is over. It’s still early, and as everyone gathers up their various computers, notebooks, and papers and leaves the room to go back to their laboratory benches to focus on their part of the huge task of attacking the virus, I notice that a few leftover bagels, the last dregs in a coffeepot and a marked-up floor-to-ceiling whiteboard are the only lingering signs we ever met.

• Jason Socrates Bardi

# Behind the Scenes

Public and Private Support  
Funds Research

## Donors Connect with Scripps Research



- 1 Fifty scientists, staff members, and supporters of Scripps Florida were guests at a January 19 cocktail reception given by Northern Trust Bank to honor Pete Hamill (left) on the publication of his book, *My Manhattan*. Here, Hamill autographs a book for Michael Bracci, president of Northern Trust Bank.
- 2 John C. Whelton, M.D., a member of the Arthritis Foundation board, and his wife Mahnaz hosted a cocktail reception in their Palm Beach home January 28 in honor of Scripps Research President Richard A. Lerner, M.D., and his wife, Nicky Lerner, M.D., Ph.D. The Arthritis Foundation has announced a \$500,000 grant to support the work of Charles Weissmann, M.D., Ph.D., professor and chair of Scripps Florida's Department of Infectology. Pictured here with Lerner (right) are friends of the Arthritis Foundation General Alexander Haig and his wife Patricia.
- 3 Scripps Florida scientists and supporters enjoyed a February 11 cocktail reception hosted by Weissmann at the Palm Beach Art and Antiques Fair held in the new Palm Beach County Convention Center. Pictured here are Scripps Research Director of Medical Education Katja Van Herle, M.D., M.S.P.H., (left) and Scripps Council of 100 member Marjorie Fink.
- 4 This year's Second Cup of Coffee series kicked off March 8 at the La Jolla Beach and Tennis Club with a presentation by Scripps Research Assistant Professor Jeffrey S. Friedman, M.D., Ph.D., on "The Role of Inflammation in Autoimmunity: A Focus on Lupus, Scleroderma, and Rheumatoid Arthritis." The series is designed for Scripps Research supporters interested in the scientific process and health-related issues. The event was co-chaired by Susan Ulevitch, Nicky Lerner, and Cleo Schimmel (left to right).
- 5 Also pictured at the Second Cup of Coffee event are donor Sharon Labovitz and Scripps Research investigator Jorge Nieva, M.D.
- 6 A \$1 million gift to Scripps Florida from George T. and Wilma Elmore (shown here) was announced at the March 11 ribbon-cutting ceremony for Scripps Florida's temporary building on Florida Atlantic University's Jupiter campus. After the ceremony, Governor Jeb Bush was guest of honor at a lunch sponsored by Wachovia Bank, attended by more than 60 senior scientists, staff members, and supporters of Scripps Florida.



## Mark Pearson: Donor with a Mission

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“Many people do not realize what a powerful, costly, and deadly disease alcoholism is, and how many lives are affected by it,” said Scripps Research donor Mark Pearson, who lost his parents to alcoholism. “I believe that with increased funding by the government and dedicated individuals, we can make advances similar to those that have been made for cancer, heart disease, and other major medical conditions in the area of alcoholism and addiction research.”

Before making his \$3 million gift to Scripps Research last year, Mark visited several academic and nonprofit research organizations across the United States in hope of finding one institution that stood out. Scripps Research was the only organization he found that focused on the neuropharmacology of alcohol addiction coupled with drug development.

“I spent time with Dr. George Koob and Dr. Barbara Mason in their La Jolla labs, and I was impressed with the research they’re conducting,” said Mark. “Dr. Koob’s dedication to understanding neurochemistry and neurobiology, and work linking addiction to stress made a lot of sense to me.”

Mark’s gift established the Pearson Center for Alcoholism and Addiction Research at Scripps Research. The center combines the latest biomedical research with innovative clinical treatment to fight alcohol and drug addiction.

“I am pleased with my decision to donate to The Scripps Research Institute,” he said, “and I plan to make additional financial commitments in this area of research in both the private and institutional sectors in the years to come.”

## Scripps Council of 100 Honors Philanthropic Leaders

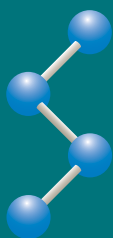
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The Scripps Research Institute has formed The Scripps Council of 100 to honor those philanthropists who, by example of their personal involvement and support, enhance Scripps Research’s reputation as an international center of biomedical research. Membership is limited to 100 individuals who generously share their time and resources, both in California and Florida, as well as contributing \$100,000 annually or making a single contribution of \$1 million or more.

Members will be invited to meet each year in Palm Springs, California and Palm Beach, Florida, where they will enjoy individualized sessions with scientists, who will inform and update them on issues, trends, and discoveries in biomedical research. These sessions will be interspersed with social events attended by institute trustees, donors, and other experts. Throughout the year, members will be invited to Scripps Research laboratories to learn firsthand the latest developments in areas of disease research of particular interest to them or their loved ones.

Founding members of The Scripps Council of 100, by virtue of their or their organizations’ contributions to Scripps Research, are: Helen Dorris; Alexander W. and Renate Dreyfoos; Richard Elkus, Jr. and Helen Elkus; Wilma and George T. Elmore; Elizabeth Fago; Marjorie Fink; Jim and Sue Gilstrap; Eugenia Glow; Wayne Green; W. Keith and Janet R. Kellogg II; Joyce Klein; Claudia Luttrell; Richard and Virginia Michaux; John and Rebecca Moores; William and Lollie Nelson; Douglas Nosworthy; Mark Pearson; Charles Scripps; Robert Scripps; Samuel Scripps; Mark Skaggs; Sam Stein; Andrew Viterbi, Ph.D., and Erna Viterbi; John C. Whelton, M.D.; and Mary Wong.

To learn more about The Scripps Council of 100 in California, please contact Denise M. Scalzo, vice president for development, at (858) 784-9365. Outside California, please contact William E. Ray, Ph.D., vice president, external affairs, at (561) 656-6401.



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