



# NEW MATH

**MENACING DISEASES LIKE TB  
COULD BE THWARTED BY  
MATHEMATICAL MODELING,  
RESEARCHERS ARE BEGINNING TO  
CONVINCE THEMSELVES. YET THE  
NEW MATH AIN'T EASY.  
BY MICHAEL FITZGERALD**

**S**ome say tuberculosis infected the first hominids. It was found in Egyptian mummies. Humans have lived and died with the bug for a long time, and it can be an ugly companion. TB has been known to destroy the lungs to the point where its victims cough blood. Its gruesomeness has been matched only by its success: TB is thought to have killed one-quarter of the adult population of Britain in the 19th century.

Fear of TB drove major reforms in social and medical policy at the turn of the 20th century, and today the disease is at record lows in the United States and other parts of the Western world. But it remains rampant elsewhere—and now it is changing in ways we don't understand.

In light of its age, it makes sense that *Mycobacterium tuberculosis*, the bug that causes TB, has a strong instinct for self-preservation. It is encased in a protective structure known as a granuloma. Treatments take six months, and TB has been learning how to shrug off those treatments. From 2003 to 2004, the percentage of drug-resistant TB cases in this country jumped up 13.3 percent. That was the highest such increase since 1993; experts wonder if a TB scourge might rise again in the West.

This ancient, and perhaps prehistoric, pathogen still infects almost 2 billion people today, a third of the world's population. We're not sure why it only kills about 2 million a year, one-tenth of 1 percent of those infected. Then again, we're not sure why it can occupy its host for 50 years—hanging out at granuloma beach, sipping mai tais (or whatever it is dormant pathogens do with themselves)—then let something else kill its host. But *Mycobacterium tuberculosis* has always had a sense of the dramatic. In 1967, Vivien Leigh suffered a recurrence of TB that would kill her. This was

and well muscled—she's a former triathlete and marathoner who still runs or cycles every single day—Flynn has an athlete's easy self-confidence. She also possesses an athlete's fondness for trash talk, and talks up her bug, TB, over the others being tackled by the new Pitt Center for Modeling Pulmonary Immunity.

"You know that TB is not the only thing being studied—even though we think it's the most important," she says, flashing a wide smile over her shoulder as she strides off to her office for a meeting.

Later, in her office, she busily arranges meetings with center researchers working on other formidable diseases. (Despite the trash talk about their chosen pathogens, she's determined that this writer meet with every single one of her center colleagues and is setting up the appointments herself.)

When she's comfortable hanging up the phone for a bit, she delves into her work using mathematical modeling to gain insight into how TB works. She started modeling more than seven years ago; that makes her

research structure than microbiology.

But Flynn made the move. That may have conditioned her for the bigger culture shift required for mathematical modeling. She's succeeding in her modeling collaborations, yet her efforts have been met with widespread skepticism from other immunologists.

"A lot of my colleagues think you can't learn anything from models, because you only get back what you put into the model," she says.

She's slowly trying to educate them on what models are good for.

She'll ask them how else they'd propose figuring out what's likely to happen over the potential 50-year period TB sits in the body.

She'll explain how models do things that can't be replicated in a lab. A model can simulate what might happen over several decades of infection, whereas a lab experiment that lasts more than a year requires tremendous effort to execute. A model can let researchers see what might happen if they knock out a specific disease protein—very difficult to do in a lab, says Flynn. A model can make it more effective to study multiple functions of cells.

## They have met with early confidence-boosting successes.

## They've also met with culture shock and the occasional crisis of faith.

just after she was in Anton Chekhov's *Ivanov*, playing the role of a woman dying of TB.

There are troves of TB data, from petri dish studies, from animal studies, from human patients, and it's next to impossible to pull all this information together.

JoAnne Flynn, however, fully intends to do so. It's her plan to develop faster treatments for TB and, by the way, a new vaccine. How? For starters, she's learning some math.

She and her colleagues are adopting a new way of thinking and talking about a handful of the world's most menacing diseases. They have met with early confidence-boosting successes. They've also met with culture shock and the occasional crisis of faith. Flynn is betting, however, that the new math will be worth all the trouble.

Flynn is a coil of energy, and in her working life, she unleashes it all on understanding TB. She holds associate professor appointments in three departments at the University of Pittsburgh School of Medicine: medicine, immunology, and also molecular genetics and biochemistry. Trim

lab a model of sorts for the other researchers involved in the new center, which is led by Penelope Morel, an MD and associate professor of immunology and medicine.

Mathematical modeling is not second nature to Flynn. She turned to it to see if it could help her pull together the legions of data on TB. But she tends to master things she sets out to do, says her husband, Mike Cascio, a biophysicist also in the medical school. (He notes that during graduate school, Flynn decided to take up basketball and developed into a solid low-post player. He says she continues to develop her cooking skills even though she's already a gourmet, and that she both ran *and* cycled every day until three kids and a good-sized lab forced her to cut back.)

Flynn trained as a microbiologist, but her interest in TB vaccines led her to pursue a second postdoc in 1990, in Barry Bloom's immunology lab at Albert Einstein College of Medicine in Bronx, N.Y. Not an easy shift.

"Immunologists don't like microbiologists," she says. "[Microbiologists] make life messy." Immunology also uses a different language and

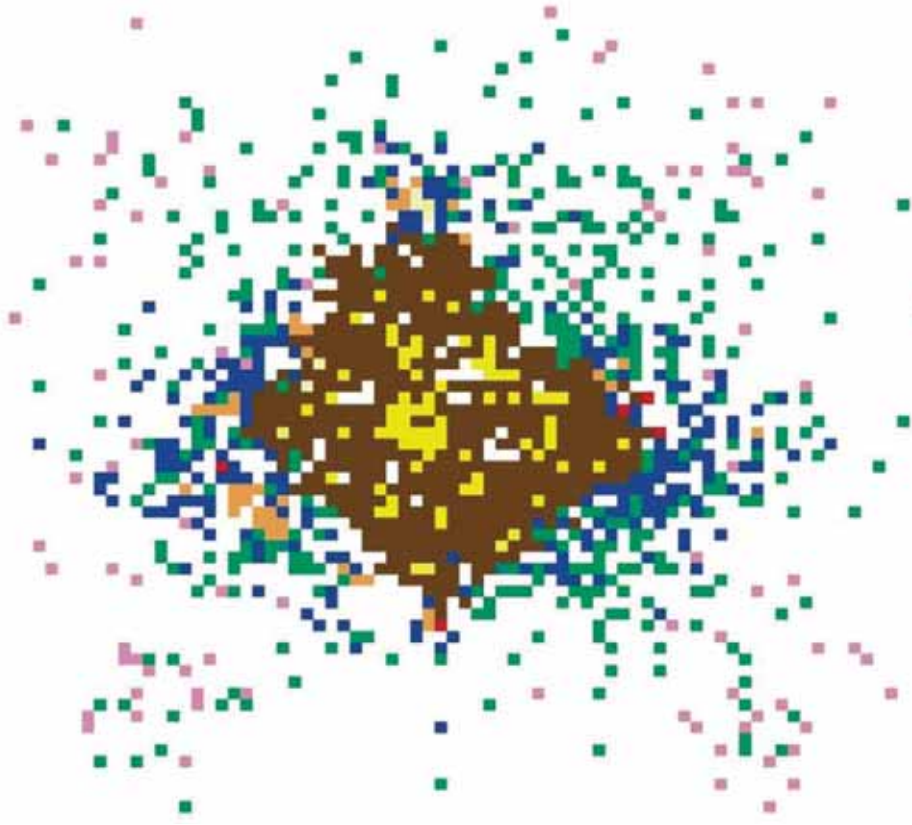
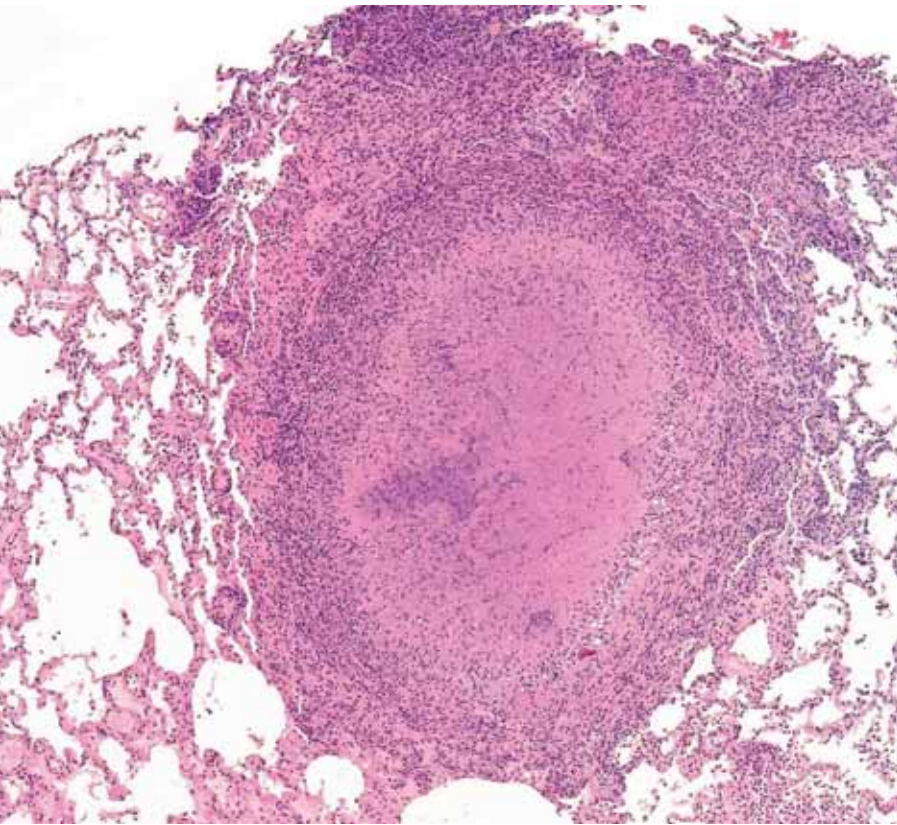
These are the kind of points she herself heard through the years from Morel. Morel—a friend and fellow foodie—has been modeling aspects of the immune system for more than a decade. Flynn would get an earful about modeling during their nights at the Pittsburgh Opera; the two have shared a subscription for years.

Flynn notes that convincing her colleagues of the merits of modeling has been a slow process, but she says, "Hey, I convinced my lab—or half of 'em, anyway."

That's a start. But what they really need, she thinks, is the chance to play with her model and her data. If they could do that, they'd get it instantly, she says.

The National Institutes of Health believes the same thing.

The agency's National Institute of Allergy and Infectious Diseases (NIAID) is giving Pitt \$9.1 million over five years so it can develop software modeling tools that will be freely available to immunologists. Pitt researchers, along with faculty members at Carnegie Mellon University and the University of Michigan, are building models of how the



immune system protects the lungs from TB, influenza, and tularemia.

The NIH is also funding Immune Modeling Centers at Duke University, the University of Rochester, and Mount Sinai School of Medicine.

Pitt might not have been on the list, if not for a dinner party back in the early 1990s, organized by Morel, the Center's principal investigator. Morel's husband, Benoit Morel, is professor of engineering and public policy at Carnegie Mellon, but he began his career as a high-energy physicist. At the time, he was teaching a course called Chaos and Complex Systems. One of Penelope Morel's colleagues and dinner-party guests asked Benoit about chaos theory and the human immune system.

Benoit Morel talks with the force of a spring torrent, and when the immunologist asked about chaos theory, the words came gushing out. The immunologist asked him to give a lecture to his class about chaos theory, and he agreed gladly.

The lecture was a dud.

"You should've seen the glazed eyes of the biologists when exposed to math," he recalls.

The Morels held fast to their belief that modeling could yield important results for immunology. Along with two colleagues, they proposed building a model to examine the behavior of Th1 and Th2 cells, immune responses that seem to antagonize each other

instead of working together.

Penelope Morel, a soft-spoken Brit, says, "I didn't think it would get funded."

But the NIH gave them funding. Their initial work led to a paper, published in 1994, demonstrating that a model was able to effectively predict how the cells would work together in the body based on amounts of interleukin-2 and 4.

The NIH renewed its grant twice, so the Morels have spent nearly 11 years modeling elements of the immune system, while gradually bringing others into the fold, like Ted Ross.

Ross is an assistant professor of medicine who also holds an appointment in infectious diseases and microbiology in the Graduate School of Public Health. He concentrates on influenza and thinks modeling could shift the flu vaccine process from reactive to proactive.

"We know that every season, flu changes. A big question is, can we develop a model to show how a virus is going to move, and can we find ways that we can build [an accurately targeted] vaccine beforehand?" Ross asks.

But mathematical models are incredibly complex things to build. Flynn and University of Michigan mathematician Denise Kirschner needed two-and-a-half years just to populate a model they could use to test ideas and find new directions for experiments. And that's in addition to time Kirschner had already

**A granuloma serves as a safe haven for tuberculosis bacteria. JoAnne Flynn and Denise Kirschner figured out how to mathematically model the structure. Flynn says this model has been a powerful tool for understanding how a TB infection can be exacerbated or controlled. (Left image shows actual animal granuloma; on right and p. 23 is the model.)**

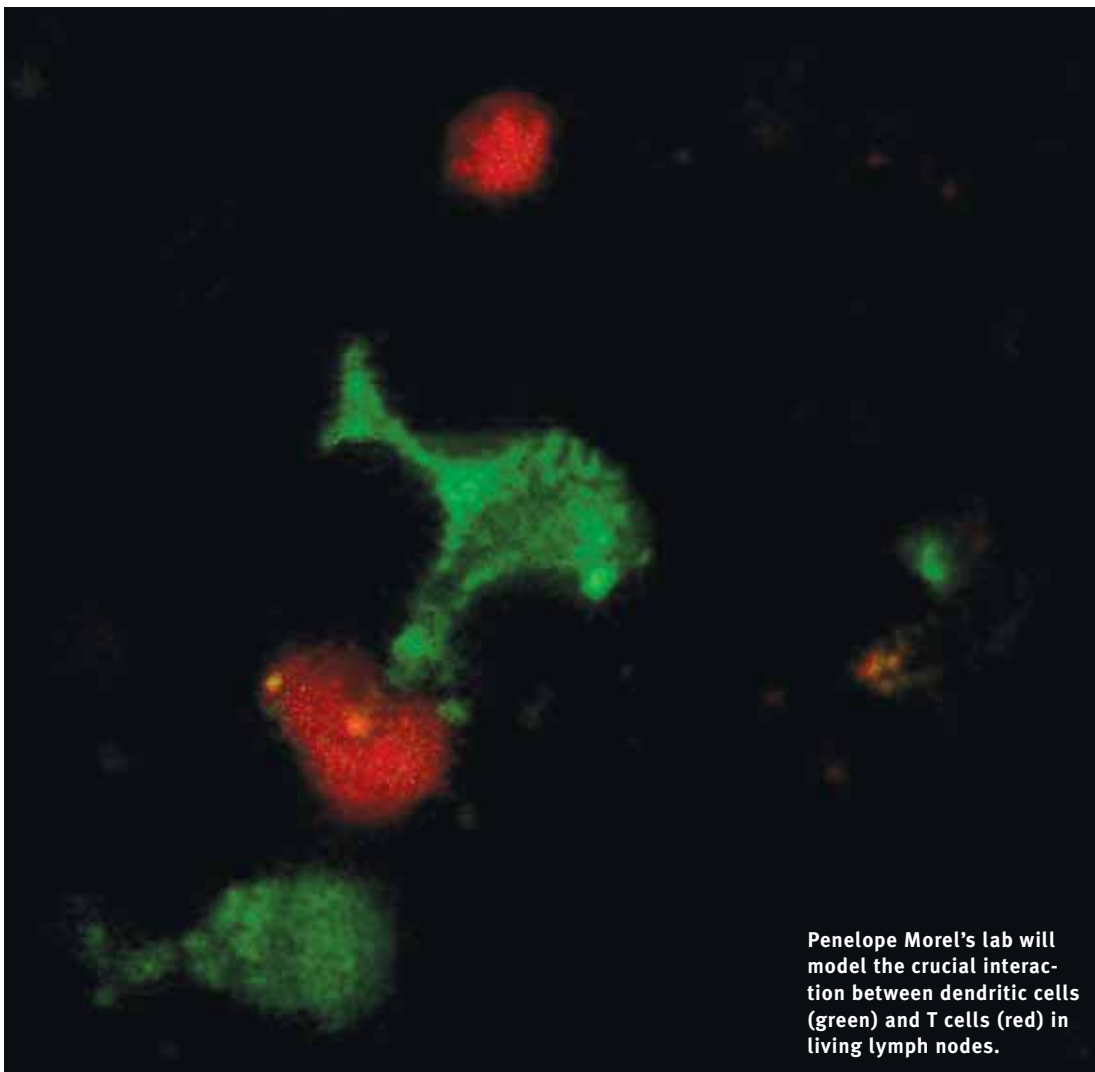
spent building a model for TB. (Her earlier model predicted that shutting down IL-10, an immune system regulator best known for its anti-inflammatory properties, would prompt TB to become active. "That's a change you'd miss in a wet lab," says Kirschner. The conclusions were published in *The Journal of Immunology* in 2001.)

Modeling the human immune system is difficult because of its remarkable complexity. Ask anyone who has tried to parse just the medical literature on TB, which is vast, despite a significant slowdown in papers published between the 1960s and 1990s.

But, ultimately, the biggest challenge in modeling comes back to Benoit Morel's initial eye-glazing lecture. Immunologists don't tend to speak the language of mathematicians and vice versa.

Penelope Morel says that often means expectations are different as well. "Mathematicians always want to know everything about everything at every single time point," she says.

"A lot of immunologists are either not willing to do those experiments, or the experi-



Penelope Morel's lab will model the crucial interaction between dendritic cells (green) and T cells (red) in living lymph nodes.

ments are just not possible to do.”

As part of the NIH grant, the various researchers gather for biweekly conference sessions, where mathematicians, computer scientists, and immunologists take turns presenting papers. The diversity of the group forces them to try to break things down—biologists don't know the differential equations, and mathematicians have never worked in labs.

Vacant stares still happen on both sides of the table.

Yet growing enthusiasm for the endeavor and camaraderie hold together the group in these still-early days. For instance, Michigan's Kirschner and Pitt's Flynn have developed a deep friendship.

Their first call went for two hours. Not only did they share research interests, they might be alter egos: They have the same cheery cadence of speech, they're both quick to laugh, and they're equally direct. They'd both recently had babies, both are unrelentingly active (Kirschner is an aerobics fanatic), and both love food.

Their early bond has been reinforced by

years of meals (sushi is the top pick) and late-night editing sessions fueled either by chocolate or bubble gum. (Both women love to crack bubble gum and are banned from doing so by their families.)

They've developed models for TB that Flynn is applying more broadly to her research. The models were key to a paper published earlier this year in *The Journal of Immunology*. That paper showed that a cell known as CD8 (cluster of differentiation 8) has a far greater role in containing TB than was previously thought. The work they've done so far will help them in the next five years, as they develop software tools that other researchers can use.

Penelope Morel is looking forward to that day: “We've got to have software to deliver so that others can use it. It's no good for us if it's just a few doctors here who are modeling.”

Flynn says once other immunologists get their hands on such software, they'll discover that modeling is “really very, very fun.” She says models “allow you to put all the things you know and a lot of things you don't know into one place.”

**Y**ou don't just stroll into a lab to glimpse *Mycobacterium tuberculosis* under a microscope. The bug is a biohazard, and the CDC requires that bench TB research take place in a Biosafety Level 3 lab.

(Level 1 labs are used for nonhazardous activities, like testing water supplies. Level 2 labs are for risky diseases like Hepatitis B. Level 3 labs are for work on life-threatening diseases that spread through the air and through communities. Level 4 classification—that's for “dangerous and exotic” aerosol agents for which there is no treatment available.)

Getting into a level 3 lab requires a skin test, a quick shot of a precursor protein to TB, followed by two days of waiting to see whether the skin swells, the telltale sign of exposure to TB.

Because TB spreads primarily through the air, working in a TB lab also requires being fitted for a mask. An industrial hygienist brings forms—there are always forms in hospitals. These probe for signs of potential breathing issues or claustrophobia. Then he pulls out an orange Kimberly-Clark N-95 Respirator Face Mask, designed to filter out aerosol particles.

You strap it onto your head, tuck it under your chin, and pinch the nose plate. He puts a hood over you and sprays a saccharin-based aerosol into the hood. You nod your head, then shake it gently. If you can't taste the saccharin, the mask works. If you can, he has a bevy of other masks. And if you count among the 5 percent of people whose faces can't be fitted, he has something that looks like a World War II-style gas mask.

Entering a level 3 lab requires more than just a mask. It means a whole new wardrobe on top of what you're already wearing: a hair bonnet, a pair of thick hospital scrubs, a set of rubber gloves taped over the wrists of the scrub shirt to seal it, a second set over that, and then two pairs of footies to cover your shoes.

You are then permitted to walk through a door with “BioHazard” warnings slapped on it. There are plenty more of those inside.

Other than sporting warning labels, level 3 labs look pretty ordinary. The one Flynn uses has four rooms with hoods, flow cytometers, and notebooks. A novel sits on a computer keyboard in one of the rooms, for whiling

away downtime during experiments. Two of the rooms serve as mouse hotels.

But there's also a bathroom. Sensible. You'd prefer not to go through the dance required to take off all those layers of protective clothing to leave the lab, just to re-suit and return a few minutes later.

The fax machine on a table in the center of the lab works well for sending notes out of the lab—a huge improvement over the days of spraying pages of notes with a decontaminant, says Amy Myers, the principal technician in Flynn's lab.

Today, Myers prepares some tests to help establish the concentration of TB sprayed at the mice for experiments. She pulls out what she calls the "Connect Four." She calls it that because its matrix of chambers makes it look like a giant version of the Hasbro game many of us played as kids. In the Hasbro game, you try to line up four disks in a row. In the Myers/Flynn edition, which includes clear rodent-holding canisters, you infect mice.

Not every mouse is exposed to the same concentration of the pathogen. The mathematicians want to know how much each mouse receives, to help them understand whether exposure levels affect infection rates. It's a reasonable question. But not an easy one to answer.

"Sometimes they don't realize how hard it is to do things they want to know," grumbles Myers.

**G**erard Nau, assistant professor of molecular genetics and biochemistry at Pitt, is a tall, slender man. He says that as an MD/PhD he was trained to bridge the gulf between the clinic and basic science.

"As great as that divide is, there's a 10-fold greater divide between biology and mathematics," Nau says.

Nau is leading research into *Francisella tularensis*, for which Pitt is building a new level 3 lab.

*Francisella* is a little-studied infectious bacterium that causes tularemia. If tuberculosis is a lumbering Saint Bernard more likely to flop down in front of the fire than bite someone, tularemia is the rabbit from *Monty Python and the Holy Grail*, savaging a human host in practically no time and often killing it. We can treat tularemia, if we diagnose it correctly. But it is also a Class A pathogen, on the fed's short list of the most-feared potential bioweapons along with anthrax, botulism, plague, smallpox, and viral hemorrhagic fevers.

Nau is no ready-made fan of modeling. He initially believed models tended to predict the obvious, like drug-resistant TB would have a global impact.

But participating in the center's biweekly conference has slowly changed his mind—thanks, in large part, to Shlomo Ta'asan, a 50-year-old professor of mathematical sciences at Carnegie Mellon who is coprincipal investigator of the modeling center.

"His approach will help me build new experiments, come up with new insights—that was an epiphany for me" about modeling, Nau says.

Ta'asan is another accidental modeler of biological systems. He's a neighbor of the Morels and says he probably first heard about modeling the immune system at one of their Christmas parties. Further conversations with his friend Benoit Morel eventually led him to do some thinking about modeling.

In 1998, Ta'asan began reading immunology books to gain the vocabulary he needed to talk to doctors and immunologists and to start developing models. Talking to researchers refined both his understanding of the science and his models.

Even so, the words don't always tell him what he needs to know. After spending months developing a model for data from microarrays, tiny chips that can process hundreds of thousands of biological samples at a time, he found that the immunologists hadn't explained a step in the experiment that involved breaking RNA into pieces. To them, it was too obvious to explain. To Ta'asan, it was disaster.

"The model was irrelevant," he says, adding, "So that was eight months!"

Frustration of this sort has sent Ta'asan into the lab. He's on a fall 2006 sabbatical, working in Penelope Morel's lab doing experiments in flow cytometry and in Gerard Nau's lab working with microarrays. Ta'asan suspects that an entirely new math needs to be developed to create the kinds of models required to understand the immune system.

"Some of my friends tell me, 'You're crazy!'" But he's hooked.

"I love this stuff. The immune system is fascinating—it's a design of, like, God or something. I'm not really religious—but wow, there is nothing else I can compare it to. This is probably the most exciting time of my career." ■

## THE WHOLE ENCHILADA

**Donald Burke has seen a lot. He studied epidemic disease while serving as a doctor in places like Cameroon and Thailand. He founded the U.S. military's HIV/AIDS laboratory and was the associate director of emerging threats and biotechnology at Walter Reed Army Institute of Research. He ran the Center for Immunization Research at Johns Hopkins University. He is an expert on avian flu, AIDS, and tropical diseases. Yet, increasingly, no matter where his work takes him, he comes back to the power of computation.**

**Burke is the new dean of the University of Pittsburgh Graduate School of Public Health. He also directs Pitt's new Center for Vaccine Research and holds an appointment in the Department of Medicine.**

**A longtime believer in mathematical models, Burke uses them to help predict the course of disease outbreaks in the general populace.**

**As an MD who came to modeling to help cure people, he thinks it is a commonsense approach:**

**"All decisions are based on models. Every time you think about a problem, you put together a mental construct."**

**Two of his main goals at the Graduate School of Public Health are to increase research on infectious diseases and boost the use of computation in public health.**

**"Computational modeling takes mental models, makes them explicit, and then helps make them more rigorous," Burke says.**

**Burke by no means thinks computer modeling holds all the answers for public health. But he does think that modeling reveals "what is unsaid, unknown, and unmeasurable."**

**His models work on the level of entire populations. They are macromodels, as opposed to the models created in labs like Penelope Morel's or JoAnne Flynn's (see p. 23 story), where the focus, so far, is on modeling molecular-level interactions. But in time, Burke intends to develop groups of modelers and medical researchers who can work together to develop a model of "the whole enchilada: the evolution of the microorganism, the immune response of the host, the behavior change of the overall population, as well as underlying social dynamics," he says. —MF**