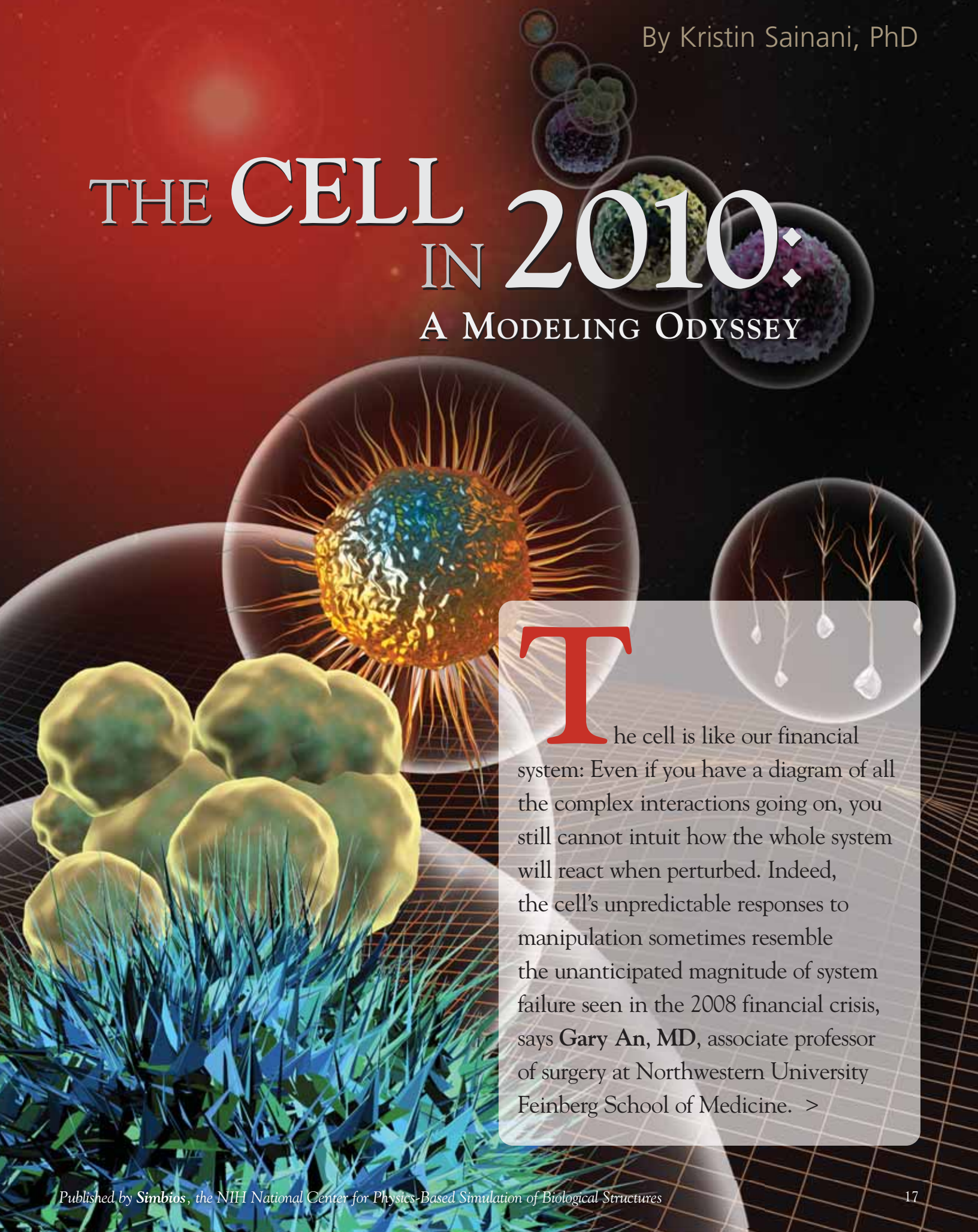
By Kristin Sainani, PhD

THE CELL IN 2010:
A MODELING ODYSSEY



The cell is like our financial system: Even if you have a diagram of all the complex interactions going on, you still cannot intuit how the whole system will react when perturbed. Indeed, the cell's unpredictable responses to manipulation sometimes resemble the unanticipated magnitude of system failure seen in the 2008 financial crisis, says **Gary An, MD**, associate professor of surgery at Northwestern University Feinberg School of Medicine. >

With hundreds of trillions of atoms, thousands of proteins, and a host of tiny organs, motors, and highways that often interact in non-linear ways, the cell is a rich target for computational modeling. But modelers and cell biologists haven't traditionally worked together. "In the past I think a lot of really interesting mathematical modeling was going on, but I'm not sure how closely tied it was to the biologists' consciousness," says **Steven Altschuler, PhD**, associate professor of pharmacology at Southwestern Medical School.

This is slowly changing. "Now is a time when both sides are realizing it's a good thing to get together. And I think a lot of progress is happening," Altschuler says.

Greater integration stands to benefit both cell biology and biomedical modeling alike.

Cell biologists need modeling to understand how genes, proteins, and pathways work together to make the cell go. "To me, it's no longer possible to even imagine thinking about these problems properly without using models as a crutch," says **Ed Munro, PhD**, assistant professor of molecular genetics and cell biology at the University of Washington. "There are simply too many moving parts and too many interactions for your brain to synthesize."

Even with relatively simple models, Munro says his intuition about what will come out of a simulation is wrong much of the time. "I'm often completely surprised," he says. "That tells me that if we're limited to assembling verbal explanations for the things we study, then we're in trouble."

At the same time, modelers need cell biologists. Traditionally, modelers have focused on either the molecular level (genes and proteins) or the macro level (tissues and organisms). But some are arguing that when it comes to multi-scale modeling, it makes the most sense to start in the middle—at the cell level. After all, molecular interactions coalesce at the level of the cell, and tissues are just a bunch of cells acting together.

"When we're thinking about multi-scale systems in biology, many people either start at the very smallish level or they start at the tissue level; I think very few people have thought of the cell as the main point. But the cell is the basic unit of life," says **Jenny Southgate, PhD**, professor of molecular carcinogenesis at the University of York in the United Kingdom.

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What follows are examples of how cell-centered models are adding fundamental insights into our understanding of cell behaviors—including how cells divide, eat, sense, move, cooperate, travel, and battle injury—as well as helping modelers bridge from the molecular to the tissue and organism levels. These models range in scale from single-cell to multi-cell, but all have implications for the basic life sciences as well as for diseases, such as cancer, heart disease, and sepsis.

MODELING THE CELL:

Beyond Biochemistry

Modelers have traditionally treated the cell as a bag of chemicals, focusing on signaling networks, such as positive and negative feedback loops. These models have led to important insights. But the biochemistry isn't happening in a vacuum; reactions unfold within, and are influenced by, the cell's heterogeneous physical environment. To truly understand cell behavior, you have to account for the physics and geometry.

"People normally think about biochemical networks and pathways. That's what systems biology is about. But, in addition to that, there's polymer physics, membrane transport, electrophysiology, electrical events, cell mechanics, and the forces in adhesion," says **Leslie M. Loew, PhD**, professor of cell biology and of computer science and engineering at the University of Connecticut Health Center, and one of the creators of Virtual Cell, a well-known cell modeling program (www.vcell.org).

"When people say that they want to model the cell, they're mostly talking about what's happening in time; very few modelers try to think about what's happening in space. And not only space, but also mechanical processes, like forces and movements," says **Alex Mogilner, PhD**, professor of neurobiology, physiology and behavior and of mathematics at the University of California, Davis.

But incorporating space and mechanics is challenging, Mogilner says. Several software programs can model simple diffusion in a relatively nice geometry, but that doesn't capture the reality of the cell. "The inside of the cell

is cluttered with all sorts of debris—cytoskeleton, organelles, and other stuff. In addition to diffusion, there's also directed transport by molecular motors. Plus, diffusion may happen in the bulk of the cytoplasm or in the plane of the membrane. It's very difficult," Mogilner says. Virtual Cell has developed the ability to model diffusion along a membrane and in complex geometries. These capabilities are state of the art.

Spatial modelers make other simplifications as well, such as modeling in two dimensions or treating cells as perfect circles. But some are trying to bridge to 3-D or account for versatile and changing cell shapes. Virtual Cell allows continuum models in 3-D; and another cell modeling program, MCell (www.mcell.psc.edu), can do discrete stochastic simulations in 3-D.

As modelers account for more and more of the cell's physical realities, it seems that, by necessity, models will get more complex and detailed. This isn't always the case, however. Models can range from all-inclusive models that attempt to perfectly mimic the cell to conceptual models that describe the cell in caricature, Mogilner says. Though it may seem that more detail would always be better, in fact there is a tradeoff between complexity and insight. All-inclusive models have a direct correspondence with experiment and tend to be more accessible to biologists and physicians, but they may add little to overall understanding.

"You can take biology, which is a big black box, and turn it into an accurate

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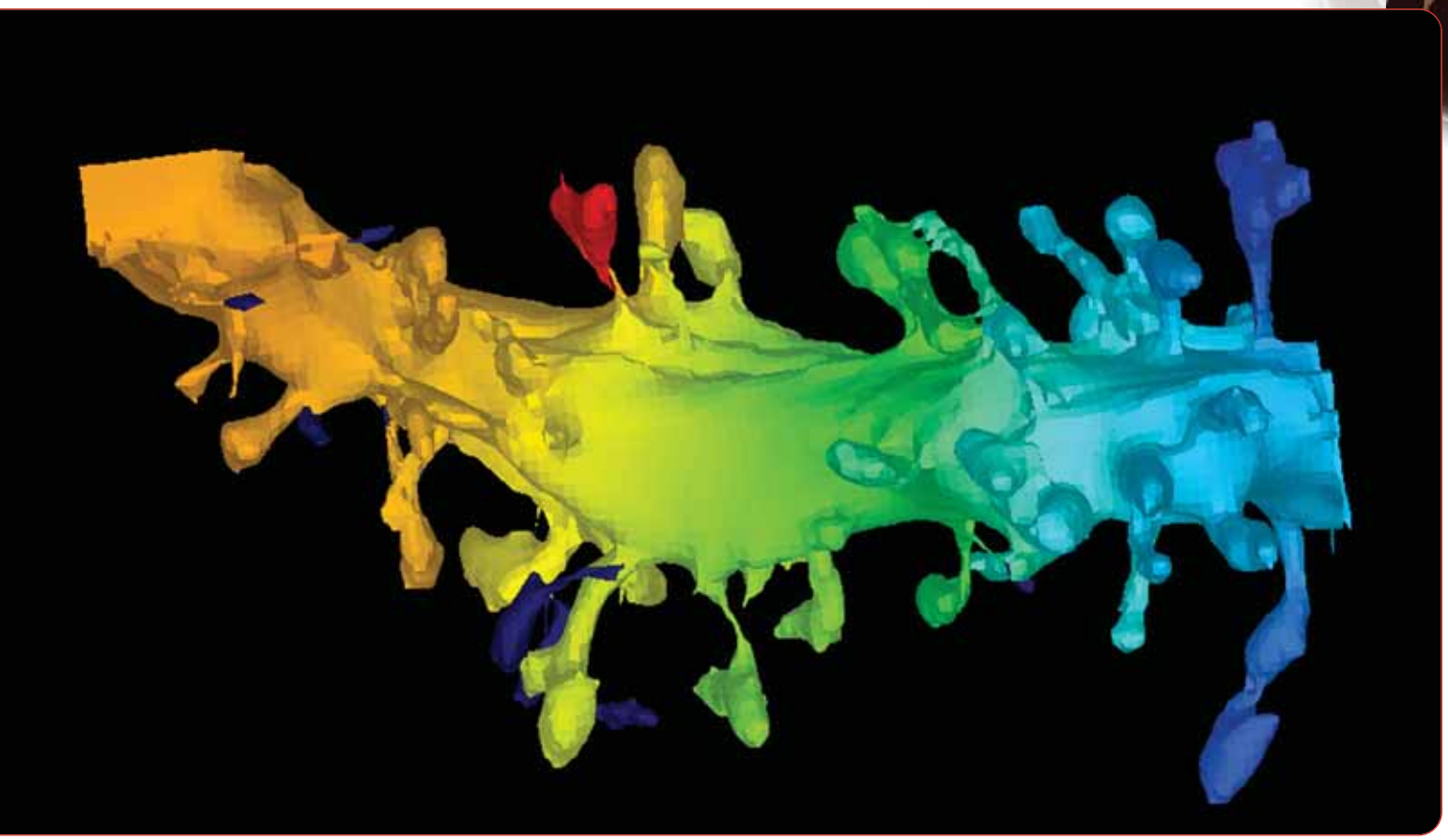

Modeling in Space. Programs like Virtual Cell allow researchers to model the spatial realities of the cell, such as diffusion on a membrane. This Virtual Cell simulation shows lipid signaling and diffusion on a protrusion of membrane on a neural cell (called a "spiny dendrite"). Courtesy of Sherry-Ann Brown, University of Connecticut Health Center; published in: Brown, S., F. Morgan, J. Watras, and L. M. Loew. 2008. Analysis of phosphatidylinositol-4,5-bisphosphate signaling in cerebellar Purkinje spines. **Biophysical Journal** ***95:1795-1812.***

simulation, which in itself has become a big black box," Altschuler says. In contrast, he says, conceptual models "give you a glimpse into something really fundamental."

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How a Cell Divides: Harnessing the Wildness of Microtubules

When a cell divides, it assembles an intricate piece of machinery called a "mitotic spindle" that physically separates the chromosomes. Chromosomes are pulled apart by filamentous rods, called microtubules, anchored on either side of the nucleus, at the centrosomes. One of the fundamental questions of mitosis is how this spindle assembles. Mathematical modeling has been instrumental in answering this question because it is difficult to experimentally follow and perturb individual microtubules, Mogilner says.

Microtubules are dynamic polymers that can rapidly shed or add proteins to their unanchored end. It's known that microtubules find the chromosomes through a "search-and-capture" process: they randomly grow and shrink from the centrosomes until, by chance, they encounter a chromosome and hook it.

In an influential paper four years ago, Mogilner and his colleagues showed that the process cannot be completely random. They built a comprehensive model of spindle assembly, including hundreds of microtubules (represented as rods that grow and shrink in different directions) and tens of chromosomes (represented as randomly oriented cylinders dispersed throughout a spherical nucleus). Their simulations showed that a purely random search-and-capture would not be fast enough to assemble the spindle in the 15 to 20 minutes it takes the cell. Instead, a "biased" search-and-capture was required—molecular motors direct microtubules to grow in areas where they are more likely to bump into chromosomes.

In a follow-up paper in *PNAS* in 2009, Mogilner's team ran simulations that probed not only the speed of biased search-and-capture, but also its accuracy. The result: there were errors in a whopping 70 percent of microtubule-chromosome attachments (for example, when a chromosome is captured by only one microtubule or by two microtubules from the same pole). In real life, cell division is highly accurate. So this revealed that the cell must use some kind of error-correction mechanism.

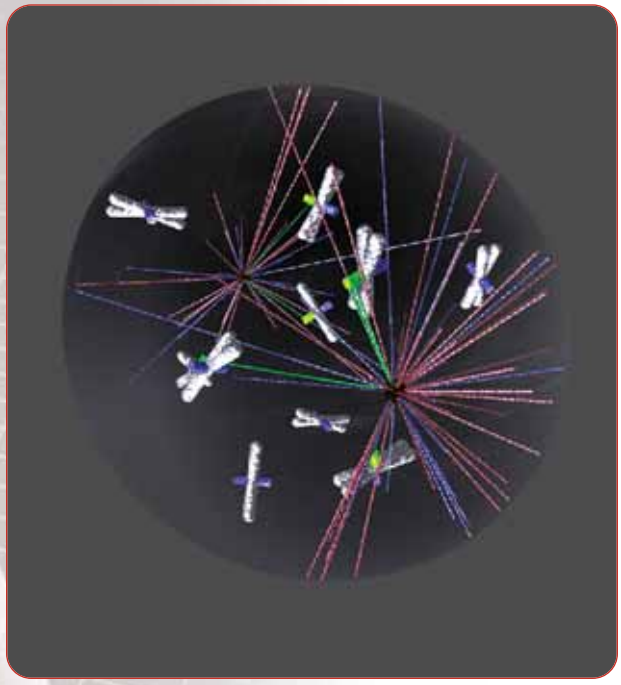
They simulated a number of plausible mechanisms but "so far, what we are finding is almost nothing can explain totally fast and accurate assembly," Mogilner says. Their model provides constraints for researchers exploring alternative error-correction mechanisms, he says.

Once microtubules have accurately captured the chromosomes, they line them up evenly at the equator of the nucleus. What's unclear is how the microtubules, which start at highly varied lengths, manage to even themselves out. "The question is: how do you harness the wildness of the microtubules, which would otherwise be inclined to grow and shorten very randomly and willy-nilly?" says **David Odde, PhD**, professor of biomedical engineering at the University of Minnesota.

In a 2008 paper in *Cell*, Odde and his colleagues used a Monte Carlo simulation to predict that an unidentified molecular motor must regulate microtubule length. Simulations showed that deleting this protein would cause microtubules to grow too long and uneven, and overexpressing it would cause microtubules to grow too short and to cluster near the poles of the nucleus. His graduate student, **Melissa Gardner**, then identified the protein experimentally: kinesin-5, a motor protein not previously recognized as a player in microtubule assembly.

The model shows that kinesin's mode of action is really simple, Odde says. The longer a microtubule becomes, the more places kinesin—which promotes disassembly—can attach to. "It evens the game out. It just keeps penalizing the ones that keep getting out ahead of the others," Odde says.

The finding has implica-



***Search and Capture.** Visualization of a computer simulation of microtubules (growing in blue, shortening in red, captured in green) searching for chromosomes during mitotic spindle assembly. Courtesy of: Raja Paul and Alex Mogilner, University of California, Davis. Reprinted from Paul, R., et al., Computer simulations predict that chromosome movements and rotations accelerate mitotic spindle assembly without compromising accuracy,* **PNAS** *106(37) 15708-15713 (2009).*

tions in cancer, as it means that anti-kinesin drugs—which are already in clinical trials—could help control tumor growth by disrupting a critical step in mitosis.

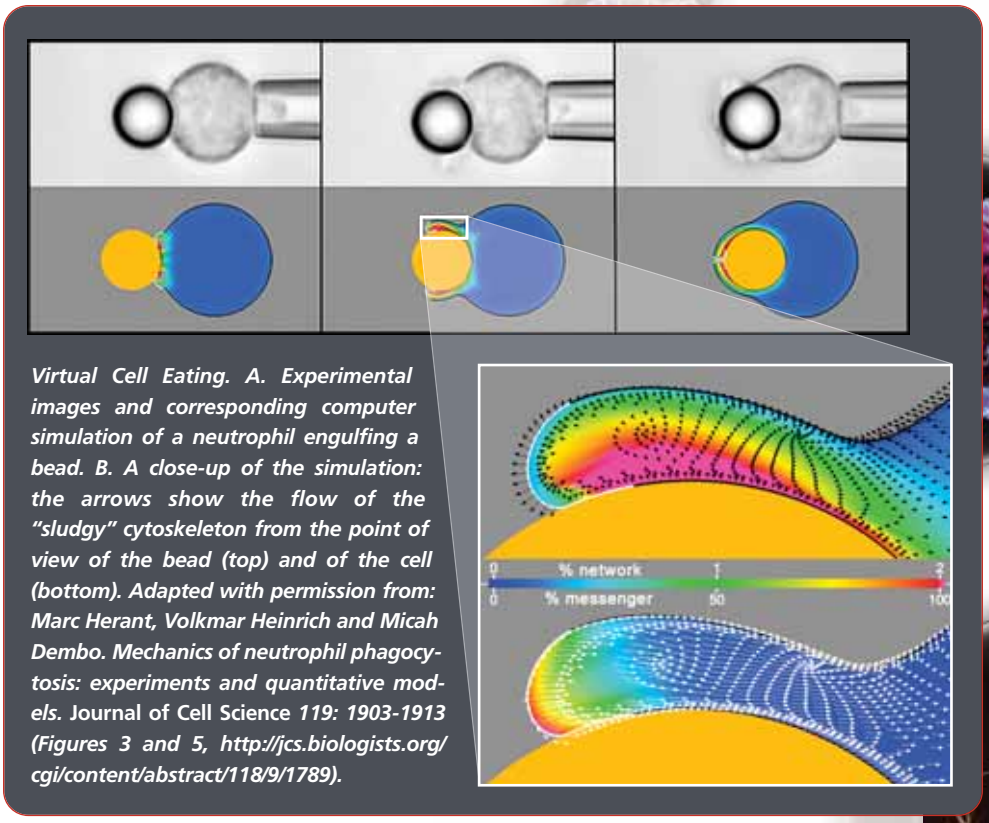
How a Cell Eats: Protruding Hands and Fingers

Single-cell organisms obtain nutrients via a process called cell eating, or phagocytosis. Using its cytoskeleton—dynamic filaments including actin and microtubules—the cell wraps itself around a particle until it's fully engulfed. Cells of the immune system use the same process to destroy bacteria and yeast and to clean up debris. "Without the phagocytosis of yeast, you would be fermented within a day or so," says **Micah Dembo, PhD**, professor of biomedical engineering at Boston University.

"Though the components of cell eating have been well worked out, mechanistic explanations are lacking," Dembo says. "We want to know: what are the forces that the cell is producing? How is the cell pushing? How hard is it pushing? Where is it pushing? Is it pulling? How does it orchestrate its little hands and fingers to do something like phagocytosis?"

Dembo has built a model of phagocytosis for neutrophils (a type of white blood cell) in collaboration with **Volkmar Heinrich, PhD**, an associate professor of biomedical engineering at the University of California, Davis, and **Marc Herant, PhD**, a research assistant professor of biomedical engineering at Boston University. Rather than model the cytoskeleton components as individual proteins or rods, "we believe at its basis, the cytoskeleton is just kind of a gooey glop," Dembo says. "It's got intermediate filaments in there; it's got actin in there; it's got microtubules in there; it's got water; it's got endoplasmic reticulum; it's got big chunks like granules and lysosomes; and the nucleus is a big rock in there. We think of it as a sludge, which, to a good approximation, can be regarded as a creeping fluid." They use a system of partial differential equations to keep track of the forces exerted by and on this viscous fluid as it moves within the cell.

In a paper in the *Journal of Cell Science* in 2006, Dembo's team reported that neutrophils use two key interfacial forces to eat a bead: a protrusive force and an intrusive force. The cytoskeleton and the cell membrane repulse each other (the protrusive force), causing a gap to open between them; as cytoskeleton polymerizes in the gap, this causes fingers of cytoplasm to jet out around the bead. At the same time the cytoskeleton and cell membrane attract each other (the intrusive force), causing cytoskeleton to build up near the membrane; as this excess cytoskeleton depolymerizes, this sucks the bead into the cell.



Virtual Cell Eating. A. Experimental images and corresponding computer simulation of a neutrophil engulfing a bead. B. A close-up of the simulation: the arrows show the flow of the "sludgy" cytoskeleton from the point of view of the bead (top) and of the cell (bottom). Adapted with permission from: Marc Herant, Volkmar Heinrich and Micah Dembo. Mechanics of neutrophil phagocytosis: experiments and quantitative models.* Journal of Cell Science *119: 1903-1913 (Figures 3 and 5, http://jcs.biologists.org/cgi/content/abstract/118/9/1789).

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Surprisingly, when the same neutrophil eats a yeast particle, it loses its ability to generate the intrusive force. "It has to slowly wrap its fingers around the yeast without any sucking in the cell is trying to grab it." The researchers don't really know why this happens, but perhaps the yeast particle has a defense mechanism that blocks the intrusive force.

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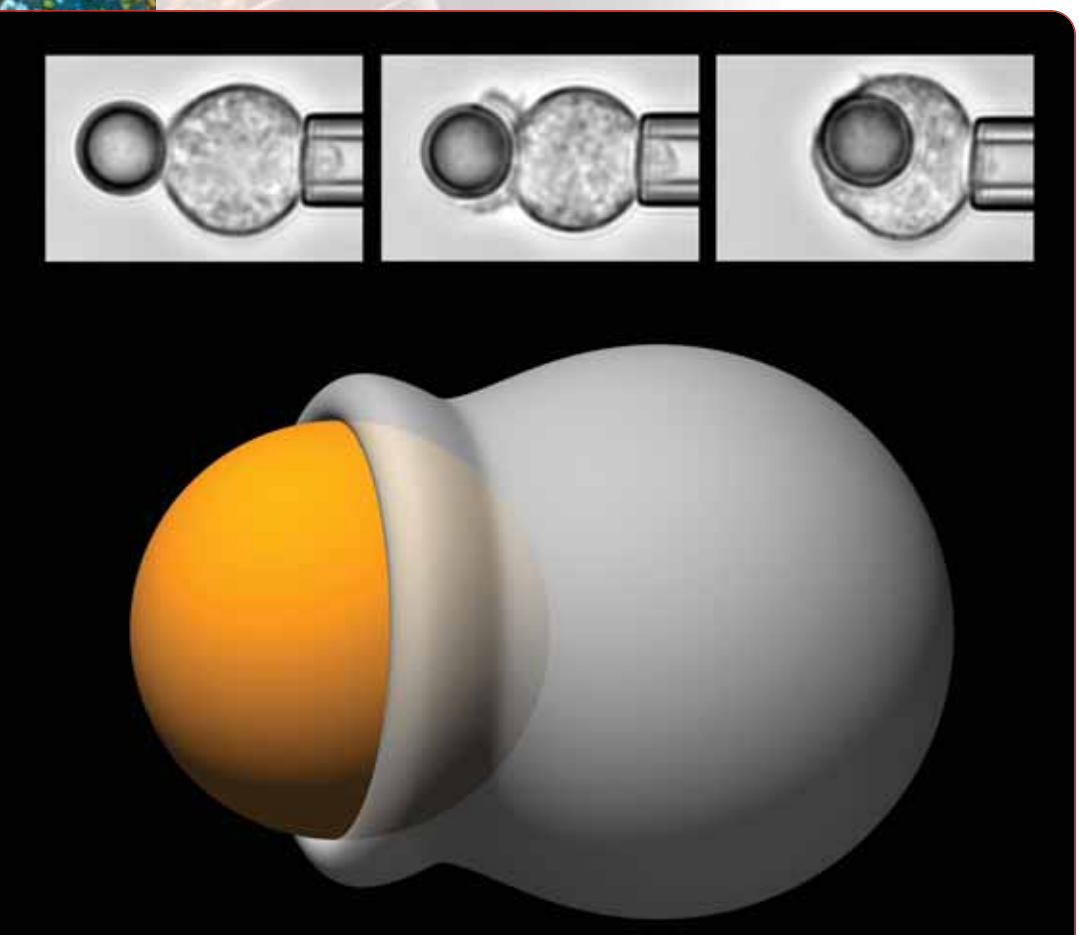
motion," Dembo says. "So the cell is trying to make a big enough hand, and it will eventually manage to do that. But in the meantime the yeast is getting pushed away [by the protrusive force] as

"I love this kind of thing because until you model it and think about it, you never realize how clever the cell is and all the problems that the poor cell is facing to do these things," Dembo says. "Without the modeling, you would just be looking at pictures of cells eating things."



Surrounded! This shows a 3D simulation of a neutrophil engulfing a bead and the corresponding experimental images. Courtesy of: Marc Herant, Boston University; Volkmar Heinrich, University of California, Davis; and Micah Dembo, Boston University.

How a Cell Senses: Feeling the Environment

The cell's environment plays a critical role in directing cell behavior. In a landmark 2006 paper in *Cell*, researchers showed that the mechanical properties of the environment alone—just its elasticity, nothing biochemical—can influence cell fate: for example, a stem cell grown on a very stiff substrate becomes a bone cell whereas the same stem cell grown on a soft tissue becomes a brain cell. Follow-up experiments showed that substrate stiffness also directly affects cell shape, motility, growth, and malignancy. "The fundamental question is: how do they sense the stiffness?" Odde says.

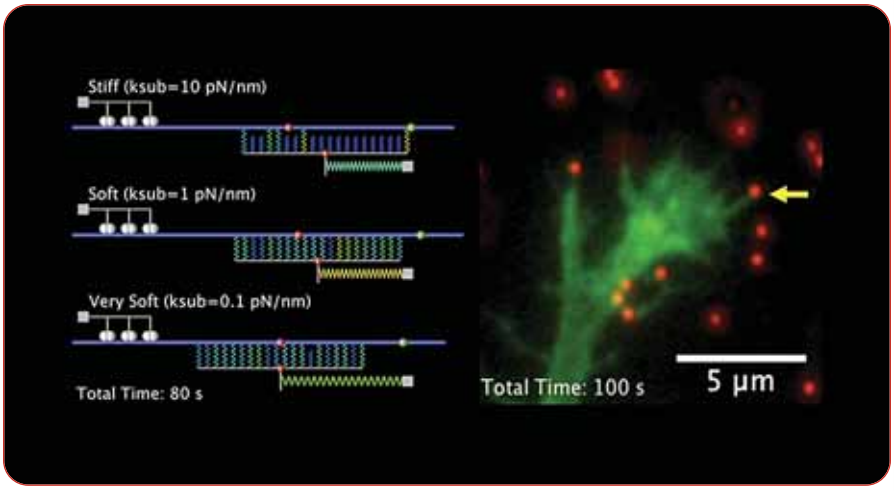
Cells bind to and interact with their environments (typically, the extracellular matrix) through proteins called integrin receptors. These receptors cluster in the cell membrane to form "adhesion complexes" that link the cell's actin cytoskeleton to the matrix and play a key role in cell movement and cell-to-matrix communication.

In a December 2009 paper in *PLoS Computational Biology*, **Daniel A. Hammer, PhD**, professor of bioengineering and of chemical engineering, and his colleagues, revealed a "simple calculation that shows why substrate elasticity affects the biology so strongly." They modeled the cell membrane and the substrate as lattices of springs and the integrins as individual springs that can diffuse along the cell membrane, cluster with each other, bind to the substrate, and pull on the membrane and substrate.

In simulations, they found that as you make the substrate stiffer and stiffer, it drives receptor clustering. "If the receptors remain distributed, then they have to pull up the substrate at many locations, and that's energetically very unfavorable on stiff surfaces," Hammer says. "What they'd rather do is get together in a cluster and then pull up the surface just in small regions."

The extent of clustering is directly correlated with cell activation. "I think the effect of substrate mechanics on cell biology is nothing more than this physical chemistry of driving clustering in these receptor patches," Hammer says.

The work has important implica-



Sensing Stiffness. LEFT: This computer simulation provides one possible explanation for how cells sense the mechanical stiffness of their environment. As myosin motors pull on actin bundles, molecular clutches (modeled as springs) engage and disengage with the substrate (also modeled as a spring). Stiff substrates have little give, and thus the clutches frequently slip and disengage; soft substrates can stretch and move with actin, so the clutches remain engaged longer. RIGHT: The motor-clutch model was tested against a series of experiments; for example, cell traction can be measured by labeling neurons (green) and soft substrates with fluorescent beads (red). Chan CE and Odde DJ, Traction Dynamics of Filopodia on Compliant Substrates,* Science*; 322: 1687-1691 (2008). Reprinted with permission from AAAS.

tions for cancer, because tumors are stiffer than normal tissues; and this stiffness promotes malignancy and growth. For example, breast tumors get stiffer and stiffer as they progress. "It used to be thought that this was an effect of breast cancer, but now people are starting to think that it might be one of the causative determinants of breast cancer," Hammer says.

In a 2008 paper in *Science*, Odde and his colleagues similarly used modeling to explore how the cell senses stiffness as it moves across a substrate. They modeled actin filaments as individual rods, and integrins and substrate molecules as individual springs. They found that more springy substrates can stretch and move with actin as the cell moves, so the clusters of integrin—which act like motor clutches—remain engaged longer. But less springy substrates have little give, and thus the clutches slip and disengage more frequently.

"So, cells, through that motor clutch system, actually have the innate ability to sense stiffness. How they actually read it out for these decisions that they make is now the next problem. And we're moving on to that and trying to apply it to brain cancer cells and how they migrate," Odde says.

How a Cell Moves: Crawling on Substrates

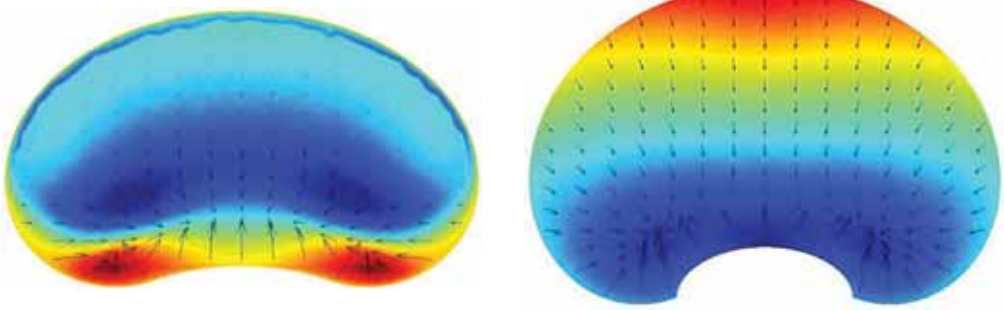
Cells move by crawling along substrates, propelled by actin filaments—which add proteins to one end and shed them from the other (called "treadmilling"). Actin polymerizes at the leading edge of the cell, pushing forward a protrusion of cytoplasm, which grabs hold of the substrate via clusters of integrins. Then the back of the cell detaches from the substrate and is pulled forward by the contraction of the actin cytoskeleton. Though the general principles are well understood, specific details are lacking; for example, it's unclear what determines a moving cell's shape and speed.

Mogilner's team devised a simple model to explain movement in fish keratocytes, fan-like cells that are among the fastest moving animal cells. "It turned out that a very simple mechanistic model, with very few equations, describes everything," Mogilner says. As actin polymerizes at the leading edge, it pushes on the cell membrane, causing tension all along the membrane (which does not stretch). This force, in turn, pushes back on the growing actin filaments. Actin density is highest in the middle of the leading edge, so the force per filament is lowest here, and actin grows rapidly. Actin density is lowest at the sides, so the force per filament is high here, which restricts polymerization. The work was published in *Nature* in 2008.

The model predicted that the higher the ratio of actin in the center to actin in the sides, the more canoe-shaped the cell would be and the faster the cell would move. These predictions were borne out by experiment.

"The equations are very enlightening because they connect the biochemistry (the kinetics of actin cytoskeleton) with the geometry (the shape) and with the physics (the forces and movements)," Mogilner says. "So I think this is a very cool thing."

Like Mogilner's model, most models of cell movement are two dimensional. This is a problem, because 3-D is not simply an extension of 2-D, says **Muhammad Zaman, PhD**, assistant professor of biomedical engineering at Boston University. In 2-D models, the cell interacts with the substrate only on one side. But when a cell moves in the body, it interacts with the extracellular matrix on all sides. "In reality a cell does not have a top or a bottom or a ventral or a dorsal surface; reactions happen all



Cells on the Go. Computer simulations of motile fish keratocyte cells. The color represents actin density (red/hot=high; blue/cold=low) and the arrows represent the flow field. The cell on the left is more canoe-shaped and moves faster due to the pattern of actin flow, whereas the cell on the right is rounder and moves slower. Courtesy of Raja Paul and Alex Mogilner, University of California, Davis.

over the surface," Zaman says. Thus the relevance of 2-D models for biological processes *in vivo* "is very limited if not completely inaccurate," he says. "More often than not, we find that the 2-D paradigms break down completely."

Unfortunately, most experiments are conducted in 2-D—on glass or plastic plates—which creates a severe bottleneck for would-be 3-D modelers.

3D Obstacle Course.
Computer rendition of a 3D extracellular matrix. The red fibers are collagen fibers that surround the cell; the cell must navigate through these during migration and invasion. Courtesy of Muhammad Zaman, Boston University.

"Modeling and experiments go hand in hand. It's very hard to publish or think about 3-D if you don't have any real data to compare it to," Zaman says. To counter this problem, Zaman's team measures cells moving through 3-D gels derived from *in vivo* sources.

Using these data, they built the first 3-D model of cell migration, a comprehensive, multi-scale model. At the lowest level, they zoom in on individual snippets of proteins in the cell and matrix, solving Newton's force equations for these snippets. "So you're looking for the right conformations that will bind, that will attach, that will stretch, things like that," Zaman says. Then they zoom out, feeding relevant information from the lower level into higher level models that solve similar force equations for proteins, protein complexes, or whole cells (with continuum rather than stochastic equations). Grid computing provides the computational power to run such large simulations.

In a 2005 paper in *Biophysical Journal*, Zaman's team explored how altering the 3-D environment affects cell velocity. Others had predicted that if you increase ligand density in the matrix—that is, give integrins more points where they can attach—this will give the cell a better grip and allow swifter motion. But, surprisingly, they showed that there is an optimal ligand density, after which speed decreases. At this point, the back of the cell experiences difficulty detaching, creating drag. "That was counterintuitive, but we showed that experimentally indeed it was the case. And the match was not only qualitatively accurate, but also quantitatively accurate," Zaman says. The validation of their computational prediction appeared in *PNAS* in 2006.

Their work may have practical implications for cancer. For example, there is a relationship between the collagen density in a woman's breasts and her chance of developing invasive breast cancer. It may be that, at optimal collagen densities, rapid cell movement increases the potential for invasion and metastasis.

MODELING MANY CELLS:

Bridging to Tissues and Organisms

The aforementioned models focus on the behaviors of single cells. But cells rarely act alone. To truly understand cell biology and to bridge to tissue and organism biology, multi-cell models are needed.

Though several approaches for multi-cell modeling are available, agent-based modeling is gaining momentum. Unlike traditional continuum models, which treat groups of cells as homogenous masses, agent-based models treat cells as individual autonomous entities. Besides capturing the heterogeneity of cells and their interactions, agent-based models facilitate collaboration between biologists and modelers.

"The cell really is an autonomous unit. It lends itself very well to agent-based modeling, where you have the one-to-one relationships between the computational model and the actual cell," says Southgate, a biologist who works closely with modelers. "For cell biologists, that's important, because you

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can immediately see the relationship between the modeling and the cell."

Rod Smallwood, PhD, professor of computational systems biology at the University of Sheffield in the United Kingdom, agrees. "Because you can talk about a computational object as if it was a physical object, this seems to make the discussions with cell biologists a lot easier. It seems much more intuitive to be able to talk about cells as if you have physical objects interacting with each other rather than to talk about sets of differential equations," he says.

Agent-based cell models also fill an important and largely untapped niche in multi-scale modeling: the middle-out model. The models can easily embed molecular-level modules, such as signaling networks—allowing them to scale down; at the same time, the collective behavior of cells falls right out of the simulations—allowing the models to scale up.

How Cells Cooperate: Growing Into Tissues

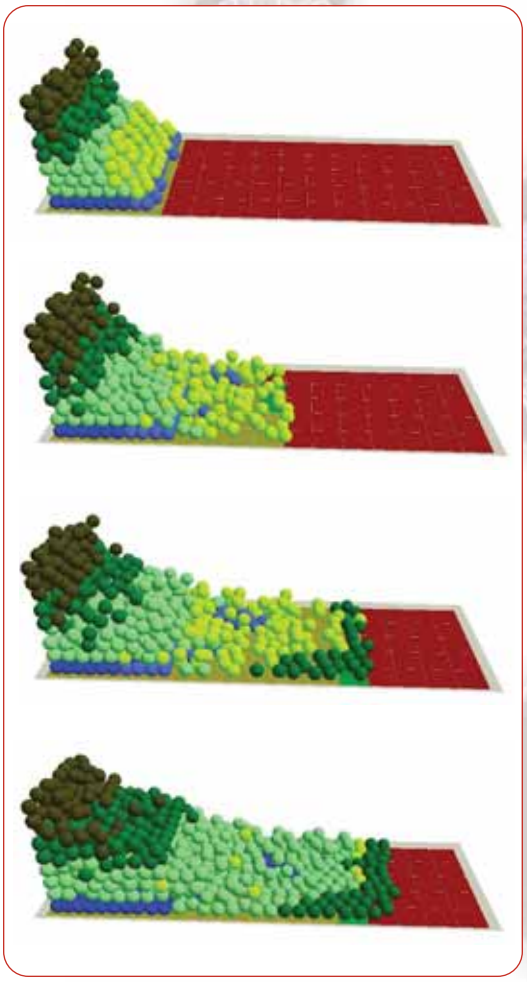
Cell cooperation plays a key role in promoting tissue growth during development and inhibiting it later in life. Cells bind to and interact with each other through surface receptors called cadherins. Mutations in the cadherins have been linked to cancer.

Southgate's team studies cell-to-cell interactions in human bladder epithelial tissue aided by agent-based modeling. In their model, rules govern whether each cell bonds to other cells, grows, divides, migrates in two dimensions, or dies. For example, each cell's probability of binding to its neighbor is proportional to the local calcium concentration. The local signaling milieu is determined by a series of mathematical models linked to the agent-based model. "We often adopt other people's pathway models, deriving rules that we then incorporate into the agent-based models," Southgate explains.

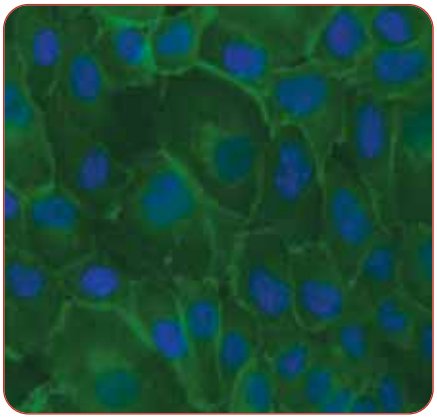
In a 2010 paper in the *Journal of Theoretical Biology*, Southgate's team introduced anti-social cells—cells lacking functional cadherin—into their models to see how they would influence normal cells and affect population behavior. In some situations, just a few anti-social cells could influence the growth of the entire population. The model illustrates one way that cancerous cells can disrupt the growth behavior of normal tissue.

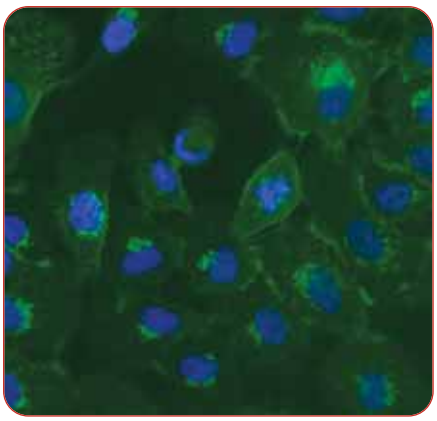
Cell cooperation is also important in wound healing. To heal a wound, cells migrate into the rift and multiply to fill the gap. The process is governed by both cell-to-cell and environment-to-cell signaling.

Smallwood and his colleagues are working out the details using 3-D, multi-scale, agent-based models. The agents are cells that can bond, migrate, divide, or differentiate. External modules determine cell signaling and resolve the forces between cells. "So there are models of particular cell signaling pathways that others have created that you can download. The functions that control cell transitions can be culled from these external models," Smallwood says. "Things move in time steps and at the end of each time step, the forces are resolved and the position and size of the cell is updated." To make the calculation computationally tractable, they model the behavior of 10,000 cells—just a fraction of the million cells involved in wound healing, but enough to capture the fundamental



***Incomplete Repair.** An agent-based simulation that shows why wounds greater than 2 centimeters across cannot heal spontaneously. Different colors represent different cell types: blue cells are keratinocyte stem cells; they change to light green as they migrate and proliferate and then to dark green as they differentiate. When the wound (red) is too big, the cells differentiate and stop moving before they can fill the gap. From: Tao Sun, Salem Adra, Rod Smallwood, Mike Holcombe, Sheila MacNeil. Exploring hypotheses of the actions of TGF-β1 in epidermal wound healing using a 3D computational multiscale model of the human epidermis.* **PLoS ONE 4(12): e8515. doi:10.1371/journal.pone.0008515.**





***Anti-Social Cells.** These bladder epithelial cells are labeled with a fluorescent antibody to E-cadherin (green), with nuclei stained blue. The top panel shows the normal pattern of E-cadherin concentrated to junctions between cells, whereas cells in the bottom panel have been genetically modified to disrupt E-cadherin and create anti-social cells. Courtesy of Jenny Southgate, University of York.*

biology, Smallwood says.

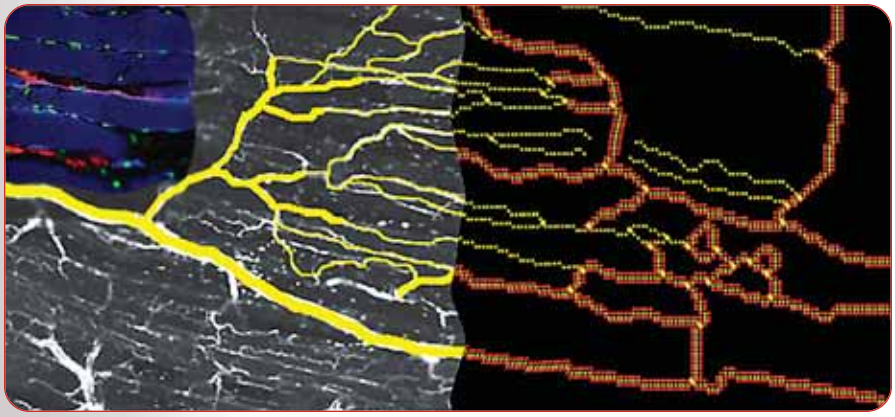
In a paper in press with *PLoS Computational Biology*, Smallwood's team used simulations to explain, for the first time, why wounds wider than two centimeters cannot heal spontaneously. The reason: cell-to-cell signaling drives the cells to first start migrating and then to differentiate; once they differentiate, they can no longer move. If the distance the cells have to migrate is too great, they differentiate before they have filled the gap. "If you can't move on any more, you're not going to heal. So that's quite interesting. You can actually see the critical reason why the wound doesn't heal," Smallwood says.

This suggests that it might be possible to get large wounds to heal if you could override the cells' differentiation rules, he says.

How Cells Travel: Trafficking in the Bloodstream

When the body is injured or invaded, immune cells travel through the bloodstream to the site of injury. They exit the bloodstream through a precise set of steps: first, they roll along blood vessel cells, then they halt to a stop, and, finally, they slide through the blood vessel wall. The process is orchestrated through adhesion molecules on both the vessel cells and immune cells (selectins and integrins), as well as signaling molecules called cytokines. A fundamental question is how cells decide where to stop in circulation.

Shayn Peirce-Cottler, PhD, assistant professor of biomedical engineering at the University of Virginia, studies immune cell trafficking with agent-based computational models. Cells drift, adhere, roll, stop, or enter tissues based on concentrations of simulated cytokines and adhesion receptors. The cells are embedded within a simulated microvascular network—complete with pressure, flow velocities, and wall shear stresses—that shuttles cells around the body. It's a complex system. The researchers have to keep track of the cells in time and space, monitoring the state of hundreds of chemokines and cell surface receptors as well as the cells' behaviors, Pierce-Cottler says. The models are two dimensional, since moving to 3-D would make them computationally intractable at this point, she says.



Traffic in the Bloodstream. Agent-based models in conjunction with* in vivo *experimental models are used to study the recruitment of circulating cells in the microvasculature of ischemic muscle. The left panel shows a confocal micrograph image of the macrovessels (yellow) and microvessels (blue and red) in mouse muscle; immune cells (monocytes) are stained in green. The right side is a screenshot from an agent-based model of this same system. Courtesy of Shayn Peirce-Cottler, University of Virginia.

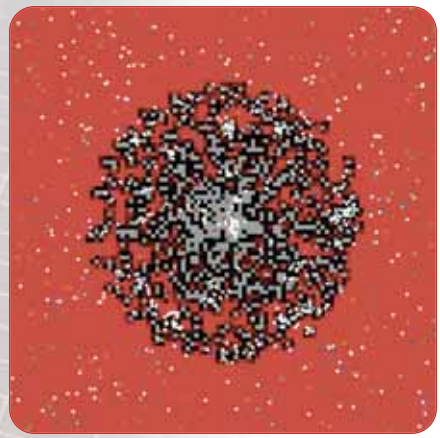
Peirce-Cottler's team is exploring the build up of plaques in the arteries (arteriosclerosis). Because inflammation is a major contributor to arteriosclerosis, it turns out that the trafficking of immune cells (particularly monocytes) to plaques plays a critical role in their initiation, progression, and eventual rupture. Peirce-Cottler and others believe that microvessels—the small blood vessels that feed into large vessels—may be an important conduit of monocytes to plaques. They are using simulations to tease out the relative contribution of monocytes from the microcirculation versus the macrocirculation.

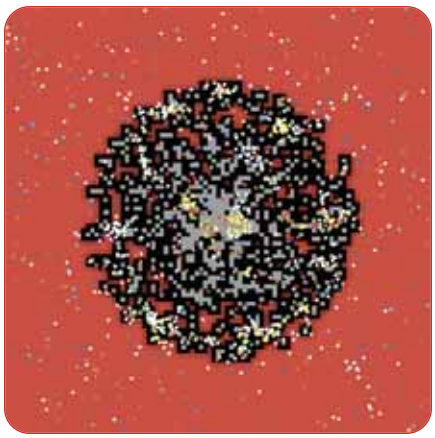
"That's hard to quantify experimentally, because you need to have a system where you're tracking individual cells *in vivo* and watching to see, when a monocyte shows up in a plaque, where does it come from. And technically speaking, we just don't have the tools to be able to do that," Peirce-Cottler says. "That's the great thing about computational models. You can actually follow an individual monocyte and say 'hey, where did you come from?'"

How Cells Battle Injury: Testing Drugs *In Silico*

A major insult to the body, such as an overwhelming infection or injury, can cause a condition called sepsis: The immune system goes into overdrive, leading to collateral damage of otherwise normal tissue, subsequent organ failure, and death. In the 1990s, researchers reasoned that since certain cytokines incite immune cells, administering anti-cytokine drugs would cure sepsis. But they were wrong. "It turns out that none of the drugs worked, and some of them actually hurt people," says Gary An, who is a trauma surgeon and ICU doctor at Northwestern University Feinberg School of Medicine.

Frustrated by these failures and the lack of effective treatments for his sep-





sis patients, An turned to computational modeling "as a means of addressing the bottleneck in translational research." It was clear that sepsis exhibited complex behaviors that could not be predicted through reductionism and linear thinking alone, he says. However, his path to computational research had a significant hurdle.

"I was not a computer science or a math guy at all; I hadn't taken anything in those areas since high school. So the computational bar was kind of high," he says. Fortunately, he discovered an agent-based modeling toolkit called StarLogo that was designed for teaching kids, and thus was very intuitive.

"The results of a cell biology paper are: I take this cell; I stimulate it with this particular compound that performs this particular function; I then see how the cell responds. Those sorts of behaviors can be converted to rules and computer code for agent-based modeling relatively straightforwardly."

He built agent-based models of sepsis and used them to run *in silico* drug trials based on actual clinical studies. The agents are the immune and blood vessel cells at the blood-to-vessel interface. The cells change states based on cell-to-cell interactions, the presence of mediators such as cytokines, and the influence of drugs. When enough of the blood vessel cells are injured, then the simulated person dies.

In a paper in *Critical Care Medicine* in 2004, he simulated what would happen if you treated populations of *in silico* patients with various anti-cytokine drugs. He showed that mortality rates were 30 to 40 percent, no better than standard treatment. He also tested different combinations of the drugs (which some had hypothesized were needed to override redundancies in the immune system), as well as various doses and durations of treatment, but nothing worked.

"By running the computational models, you identify that the disease state itself is very, very stable and resistant to change," he says. "When you simulate the intervention, you get this sort of pebble in the stream effect where you might see a little bit of a result initially, but the flow of the system is such that it basically swallows up your intervention and it doesn't have any effect."

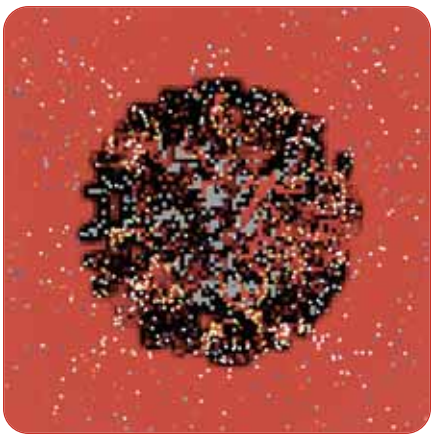
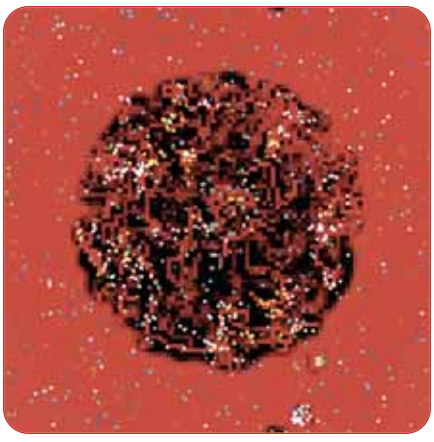
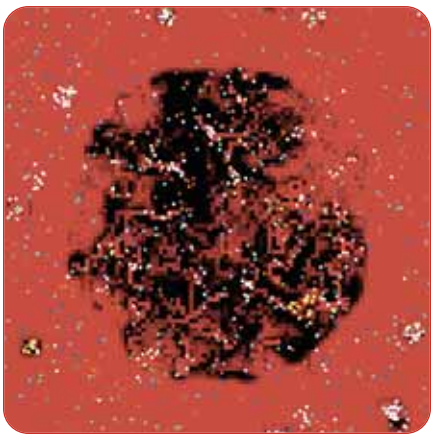
"System-level computational models are invaluable in identifying these types of unexpected behaviors, and will play a critical role in addressing the challenges of developing effective therapeutic interventions," An says.

"System-level computational models are invaluable in identifying these types of unexpected behaviors, and will play a critical role in addressing the challenges of developing effective therapeutic interventions," Gary An says

Bringing Modeling and Cell Biology Together

Despite these recent successes in pairing cell biology and computational modeling, the two fields remain only loosely integrated. Breaking down these barriers will take long-term collaborations, Zaman says. For example, his lab comprises half experimentalists and half modelers. Yet, he says, "I still see it in many of my students that it takes a long time before they can speak a common language."

"We need a more integrated environment, not only for the computations to be more powerful, but also for the experiments to be more probing and much more quantitative," Zaman says. "I think the burden of responsibility is on both sides." □

Sepsis Explosion. (Lower opposite page and below) These serial screenshots from a 2-D agent-based simulation of inflammation and sepsis follow the progression from infection, to initial immune response, to cell death and the start of healing. Upon infection with bacteria (gray areas), the healthy blood vessel cells (red) become damaged (dark red) or die (black). Gradually, inflammatory cells (white neutrophils) gather near the bacteria and become activated (yellow or other colors). The inflammatory cells gradually clear the bacteria, allowing healing to occur. Courtesy of Gary An.