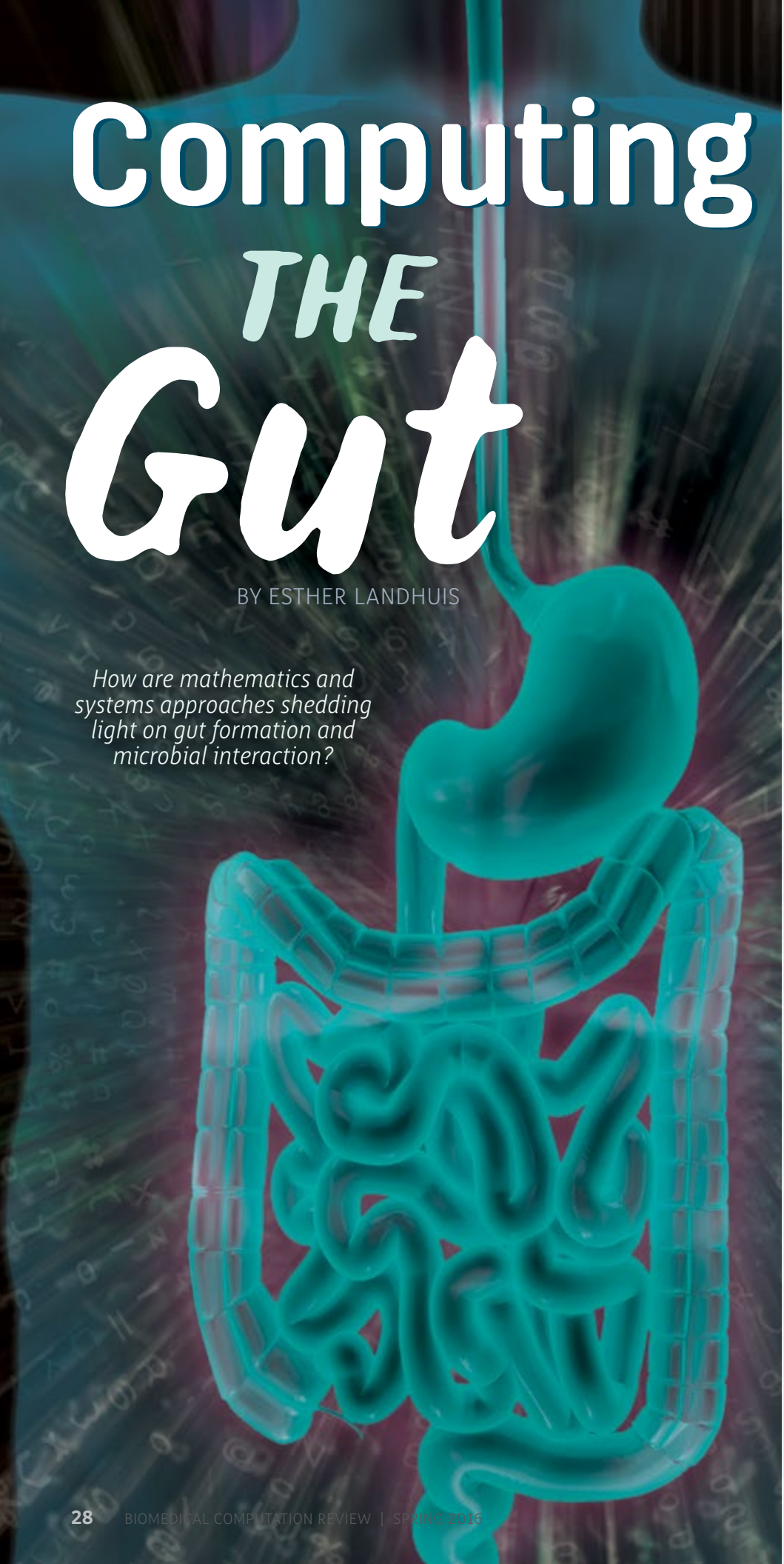
Computing THE Gut

BY ESTHER LANDHUIS

How are mathematics and systems approaches shedding light on gut formation and microbial interaction?



The heart holds a special place in human history and literature, and the brain may be the organ we most associate with a sense of self. But the proverbial seat of wisdom—the gut—deserves reverence, too.

It is an architectural wonder buzzing with activity. A 20- to 40-foot tube with many tight bends and folds, the gut houses trillions of bacteria working in cahoots with our own cells to extract energy from food and maintain health.

How does this long tube cram inside the belly without becoming a tangled mess? Why doesn't food get stuck in there? And what about all those bacteria? How do they work with gut immune cells to keep us from getting sick?

The sheer complexity of the gut—and the finger-like projections called villi that line the intestinal tissue—is inspiring some scientists to explore how physical forces, such as changes in stress or geometry, influence how the gut is formed. In addition, a growing suite of mathematical models and computational tools is offering insight into how immune cells within this engineering wonder interact with native bacteria and foreign pathogens to regulate health.

Gut Formation: Loops, Wrinkles and Folds

So how does nature design a gut?

"If you took a garden hose and randomly folded and packed it, you would form kinks—and this would be problematic," says **Thierry Savin, PhD**, a biophysicist at the University of Cambridge. "Yet nature has designed a smart, elegant way to put regular loops in the gut without forming kinks."

As a former postdoctoral researcher at the School of Engineering and Applied Sciences at Harvard University, Savin collaborated with Harvard mathematician **Lakshminarayanan Mahadevan, PhD**, and developmental geneticist **Cliff Tabin, PhD**, to explore the physics behind this amazing feat.

Tabin and colleagues had examined dissected embryos of chicks, quails, finches and mice, and seen that the gut forms loops that are strikingly similar in number, size and shape across species. When his team surgically separated the gut from the rest

of the embryonic tissue, the loops remained intact. However, if they cut the gut tube away from its attached membrane, the looping structure disappeared—the tube relaxed into a straight configuration and the membrane shrank. The big question, says Savin, was, "How do you form this shape? What is the strategy nature uses to make the loops?" Could it be that the tube grows faster than the attached membrane, which gets stretched and forces the gut to coil?

Experiments with common lab materials gave the team a sense for how this might play out. They stitched a straight rubber tube to a stretched latex membrane, then let the structure relax. It spontaneously adopted a helical pattern that looks like the biological gut. What happens at the scale of a single loop is the same as what happens with a taut bow. "If you cut the string, it becomes straight," says Savin. "This convinced us that elastic forces originating from differential growth between the tube and membrane are responsible for shaping the gut."

Savin and his colleagues produced a graphical simulation of gut looping in a chick embryo using a model based on geometry, the mechanical properties of the tissues, and the relative growth rate of the gut tube and the mesentery (bottom). The simulation compared favorably with both the rubber model (middle) and an actual chick gut (top). Image courtesy of T Savin and A Shyer.

Further experiments with the rubber-latex structure helped the researchers work out mathematical equations to account for altering specific parameters—for instance, membrane stiffness, tube size and radius—to produce distinct looping patterns in the gut. The team made similar measurements in gut tissue from chick, quail, finch and mouse embryos at various stages of development to refine and confirm their mathematical model.

More recently, Tabin, Mahadevan and colleagues extended their modeling to incorporate genetics. In a 2015 *Cell* paper, the researchers report how mechanical forces in the developing gut activate molecular signals that position intestinal stem cells at the base of villi, where they give rise to the other cell types in the gut lining.

Another group of interdisciplinary researchers has also used mathematics and computational tools to examine gut formation. However, rather than study the looping structure of the gut, they focused on the formation of epithelial patterns during embryonic development of the gut's inner layers—the endoderm and mesoderm—by modeling them as concentric tubes. The work by **Pasquale Ciarletta, PhD**, an applied mathematician at the Université Paris 6 and Politecnico di Milano, **Valentina Balbi, PhD**, Ciarletta's graduate student at the time, and **Ellen Kuhl, PhD**, a bioengineer at Stanford University, was published in December 2014 in *Physical Review Letters* and February 2015 in the *Journal of the Mechanics and Physics of Solids*. Their model explains how the tubes' elastic and geometric properties influence wrinkling and folding patterns in the epithelia of the esophagus, intestines and other gastrointestinal tissues—traits that contribute not only to development but also disorders of the intestines, such as food allergies.

The team started by collecting existing experimental measures of the thickness, elasticity and growth rate of the gastrointestinal tract of chick and turkey embryos at different stages of development. From these measurements they calculated parameters that drive key pattern transitions during development. For example, certain geometric and mechanical properties triggered development of ridges on day 13 and caused villi to form on day 14, Ciarletta says. The model explained how the esophagus develops longitudinal folds with a thick and stiff outer layer, while circumferential folds emerge in the jejunum with a thinner and softer outer layer.

Researchers can use the model to explain and predict changes in gut morphology that lead to digestive disorders. In people with food allergies, for example, local inflammation can cause atypical wrinkling that is a hallmark of disease.

Insights from modeling point toward potential treatments that tweak the tissue's mechanical properties—for example, osmotic drugs to restore the homeostatic condition, Ciarletta says.

Gut Microbiome Variation

Moving beyond architecture, some scientists are developing computational methods to survey the constituents of the gut—specifically, its cells and microbes. Our bodies have about as many microbes as cells, and microbiomes vary dramatically between individuals. With advances in genomic sequencing and analytical methods, researchers have compared samples of gut bacteria from different people and found vast differences in which species are present and which genes they encode.

Research suggests that microbiome variation may influence many aspects of health. Gut bacteria shape immune system development and can affect how well we digest certain foods and how easily we gain weight, research suggests.

Yet gut microbes aren't the whole story. "It's not only which players are there but how they interact with each other and with the host. It's important to study [the gut] as a complex system," says **Elhanan Borenstein, PhD**. A computational biologist, Borenstein runs a lab in the Department of Genome Sciences at the University of Washington in Seattle. His group hopes to gain an improved, systems-level, mechanistic understanding of the microbiome using systems biology approaches and computational modeling.

One question that intrigued **Sharon Greenblum**, **PhD**, during her graduate studies in the Borenstein lab, was the extent to which the gut microbiome varies across individuals at the strain level. This information could be important because different strains of the same species of bacteria could encode different genes and may therefore perform different functions in the gut. They might also have more or fewer copies of particular genes.

Many studies of the gut microbiome use methods that are not sensitive enough to characterize the bacteria at the strain level. To study strain-level differences, Borenstein and Greenblum, who is now an evolutionary genetics postdoc at Stanford, used a different approach. Their method involved sequencing short stretches of DNA in the sample and counting how many map to a specific gene in a particular species. They used this method to analyze gut microbiome data from previously published studies of fecal samples from healthy, obese and inflammatory bowel disease (IBD)–afflicted people.

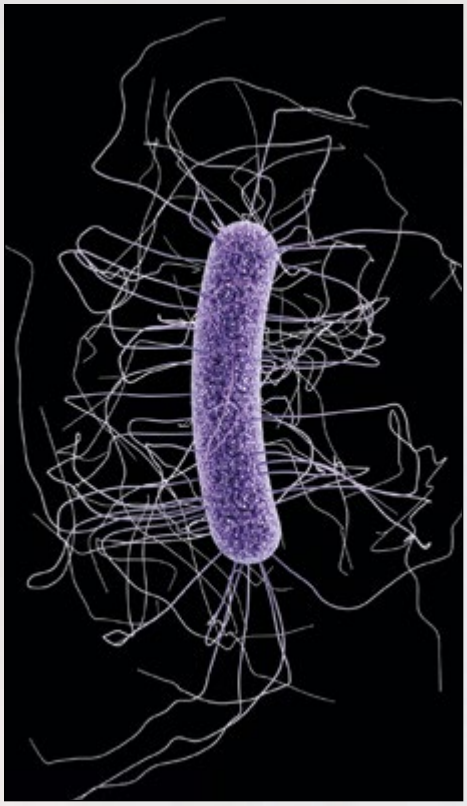
First they had to determine whether a gene was more abundant in a particular individual simply because the sample contained a greater number of species each encoding the gene, or because that individual's strain of the particular species contains more copies of the gene. Indeed, the team wondered: When comparing individuals with the same bacterial species, could one person's strains have more copies of a certain gene while another person's strains have fewer copies?

To address these issues, the researchers developed algorithms to map each shotgun sequence to the bacterial genome it came from and determine— for each sample, and for each bacterial genome in that sample—the copy number for each gene. Next, they compared between samples, asking if the copy number of gene X in species Y is the same as it is in other samples. The goal was to identify cases where a specific gene in a particular species is present in different numbers of copies across individuals.

As reported in February 2015 in a *Cell* paper, there was "tremendous variation" among individuals, Borenstein says. For most species analyzed, individuals had copy number differences in many genes. Moreover, for some genes, one person could have a single copy while another had 15.

"We were surprised by the amount of variation," Borenstein says.

And copy number variation did seem to impact function—particularly for genes associated with responding to the environment, such as those encoding proteins that transport metabolites in and out of cells. This makes sense: In a nutrient-poor environment, a higher copy number of a specific set of transporters might be advantageous for feeding the cell, Borenstein notes.



This illustration, which is based on photomicrographic data, depicts the morphology of a single Clostridium difficile *bacillus, a common cause of antibiotic-associated diarrhea. Over the past several years nationwide, states have reported increased rates of* C. difficile *infection, as well as more severe disease symptoms and an associated increase in mortality. Credit: Centers for Disease Control/James Archer.*

By quantifying the extent to which the gut microbiome varies between individuals, Borenstein and his colleagues have taken a first step toward the lab's eventual goal: personally tailored interventions. "We want to be able to design a specific perturbation to create a specific phenotype," Borenstein says.

Today, to coax a patient's microbiota toward a healthy microbome composition, physicians use fecal transplantation: They take a stool sample from a healthy person, "transfer it into a diseased person and hope it works," Borenstein says. But Borenstein's lab is striving for rational design rather than trial and error. And to achieve that, he says, "We need to build accurate, mechanistic models of the microbiome."

Tackling Superbugs

Broadening the analyses further, some research groups are building computer models to study dynamic interactions within the gut—not just among microbes but also the immune cells that live and work alongside. The body's ability to fight dangerous pathogens depends on coordinated interplay between microbial and immune systems, each consisting of diverse cell types. Sometimes a menacing microbe can throw this network out of whack. One such culprit is *Clostridium difficile*—a "superbug" that infects some 600,000 people in the US each year, killing 29,000. Healthcare costs associated with *C. difficile* infections top $3.2 billion. Worse yet, these numbers are on the rise.

So it may be disconcerting to learn that *C. difficile* are actually found everywhere. They can even live in a normal human gut—though "usually in low quantities and kept in check by good bugs," says **Steven Steinway**, **PhD**, an MD/PhD candidate at Pennsylvania State University working with biophysicist **Reka Albert**, **PhD**, also at Penn State, and biomedical engineer **Jason Papin**, **PhD**, at the University of Virginia.

C. difficile only becomes a problem when antibiotics prescribed to fight one infection deplete the body of other bacteria, many of them beneficial. That gives superbugs a chance to grow and dominate—which calls for another round of therapeutics. "What's ridiculous is that *C. difficile* infection (CDI) is caused by antimicrobial treatment, yet the treatment for CDI is another set of antimicrobials," says **Josep Bassaganya-Riera**, **DVM**, **PhD**, director of the Nutritional Immunology

and Molecular Medicine Laboratory (NIMML) at Virginia Tech in Blacksburg. "There is an unmet clinical need for safer and more effective therapeutics for CDI, and modeling can accelerate the development of such new treatments."

As described below, these research teams are using computational tools to find strategies for tackling *C. difficile* infection—one focused on finding good bacteria to do the job and the other focused on boosting immune defense.

Beneficial Bacteria

As reported in *PLoS Computational Biology* in June 2015, Steinway and his collaborators modeled metabolic interactions in the gut microbiome in order to identify specific bacterial strains that act to suppress *C. difficile* growth. His team hopes the insights will lead to the development of probiotics to supplement conventional antimicrobials for people battling a CDI.

Steinway says the model views the intestinal community as an ecological niche—sort of like a rainforest—with diverse organisms that interact in predator-prey relationships. However, in a microbial community, the bacteria are not necessarily preying on each other but "produce chemicals that can help or suppress the growth of other bacteria," Steinway says.

His team's mathematical model was built from mouse data showing that treatment with the antibiotic clindamycin makes animals more susceptible to *C. difficile* infection relative to untreated controls. The researchers measured quantities of different bacteria in the mouse gut and monitored changes in these populations over time.

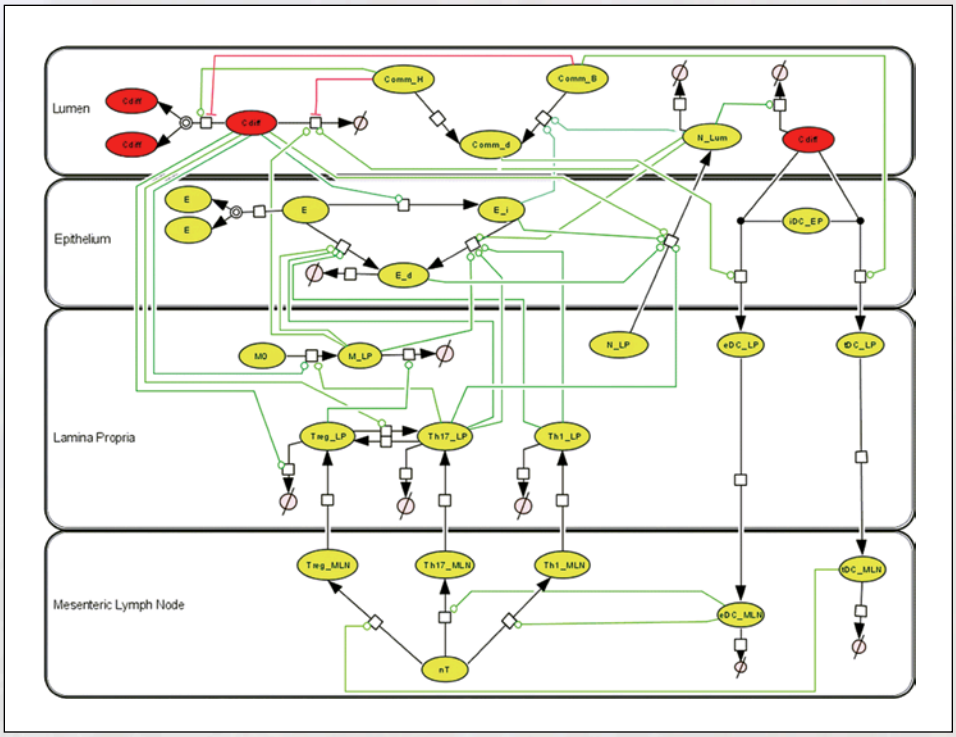
To model cause-and-effect relationships among bacteria, the team used a binary approach: For each timepoint in the mouse data, the researchers determined which bacteria were present and which were absent. These data were then crunched by machine learning algorithms to reveal which strains were likely activating or inhibiting other bacteria.

Their model identified a strain of normal gut bacteria, *Barnesiella intestinihominis*, that inhibits *C. difficile* growth—a result that has been confirmed by lab co-culture experiments. If the tests pass muster in mice, the team hopes to move toward human trials of the probiotic.

An Immune Boost

Rather than identifying good bugs to counteract *C. difficile*, Bassaganya-Riera's team sought to understand how to help specific immune cells do a better job of keeping the superbug in check. To figure that out, they needed to know how *C. difficile* disrupts the balance between the branch of the immune system that promotes inflammation (the effector branch) and the regulatory branch that suppresses it. This question is hard to address with traditional experimental approaches because the relationships among the players are networked rather than uni- or bi-directional.

The team therefore turned to computational modeling to explore interactions between pathogens and the host's gut bacteria and immune cells. They began by modeling a network of 23 interacting entities across the four-part architecture of the gut's mucosal immune system: the lumen—the inner part of the intestine—where beneficial bugs and pathogens are located; the epithelium—the layer of cells that separates the body from its external environment; the layer beneath that, called the lamina propria, where most immune cells reside; and lymph nodes, where immune reactions begin. To build and calibrate the model, they used data from immune cell populations analyzed individually in *C. difficile*–infected mice over the course of an infection. The model relies on ordinary differential equations to describe the cell dynamics during



Bassaganya-Riera and his colleagues created a network model of the gut immune response to Clostridium difficile *infection (*CDI*) across four compartments of the intestinal mucosa (black boxes) as diagramed here. In the model,* C. difficile *interacts with other bacteria as well as immune cells in various ways. For example, interactions could activate the bacterium to start proliferating—or inhibit or kill the bacterium. Alternatively, interactions could modify various other reactions among the participants. Species include* C. difficile *(Cdiff, in red), infection-exacerbating commensal bacteria (CommH), protective commensal bacteria (CommB), dead commensal bacteria (CommD), epithelial cells (E), inflamed epithelial cells (Ei), neutrophils (N), macrophages (M), dendritic cells (tDC and eDC), T cells (nT, Treg, Th17, Th1) existing in multiple compartments: lumen (Lum), epithelium (EP), lamina propria (LP), and mesenteric lymph node (MLN). Reprinted from A Leber, M Viladomiu, R Hontecillas et al., Systems Modeling of Interactions between Mucosal Immunity and the Gut Microbiome during Clostridium difficile Infection,* PLoS One, *DOI:10.1371/journal.pone.0134849 (2015).*

the infection as well as the effect of the bacteria-killing chemicals some of the cells were producing.

And as it turns out, churning out such chemicals—or antimicrobials—wasn't necessarily a good thing. Secreting more bacteria-killing compounds did not dampen CDI but rather sustained it by preventing regrowth of beneficial bacteria that could have quashed the superbug. "A significant amount of damage during CD infection is not caused by the pathogen itself but rather by the overzealous host immune response," Bassaganya-Riera says.

Published in July 2015 in *PLoS ONE*, the model is steering the researchers' attention toward therapeutic responses that manipulate the host rather than the bacterium. The goal: to allow the host immune system to co-exist with bacteria such as *C. difficile*, Bassaganya-Riera says.

His team will now begin testing the model's predictions in his multidisciplinary lab. "We have computer scientists, mathematicians, and physicists but also immunologists and lab technicians," Bassaganya-Riera says. Penn State's MD/PhD program and Virginia Tech's NIMML create "researchers who can navigate the interface between experimental and computational work—that is, spend the morning writing code and in the afternoon perform studies in mice or analyze clinical specimens."

Gut Tissue Modeling

Gary An, MD, associate professor of surgery at the University of Chicago, also straddles multiple disciplines. An trained as a trauma surgeon in the mid-1990s but grew frustrated by decades of failed attempts to develop treatments for sepsis—a life-threatening illness caused by disordered systemic inflammation. Around that time he learned about complexity science and agent-based modeling, an emerging approach for studying systems with interacting components that can behave in unexpected ways.

In the entertainment industry, such models are used to create virtual worlds in video games and movies—for example, battle scenes in *Lord of the Rings*—where individuals operate under similar guidelines yet behave differently moment to moment, leading to unanticipated outcomes for the group. An considers cellular interactions within the human gut an analogous situation. "One of the huge advantages of agent-based models is the ability to construct spatial representations that look real," An says. "This is why it's used for battle scenes in movies. That's why it's used to model birds flocking and traffic and things that have a spatial pattern to them."

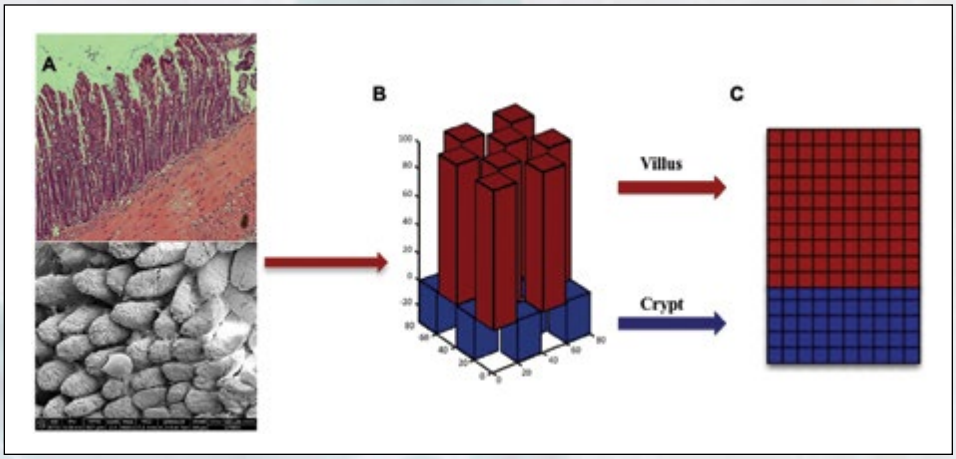
Just as birds arrange into a flock, "Tissue forms a certain structure because of the cells' interactions," An says. "My emphasis on the models is their ability to generate tissue architecture." This is important because histology—the study of tissue slices under a microscope—is a primary means by which physicians diagnose and characterize disease.

Consider for example ulcerative colitis, a disease in which the gut surface becomes unusually sensitive, leading to dysregulated inflammation and painful ulcers in the digestive tract. Some cases are treated by removing the colon and folding a piece of the small intestine to form a stool-collection pouch. The problem is, "the pouch can become inflamed and make people sick," An says.

An's team suspected that the resulting condition, called pouchitis, is caused by inflammatory signaling from high bacterial counts in the accumulating stool. If so, clues to detect the transition from normal to pathological could appear as shifts in the gut's tissue architecture. In March 2014, An and colleagues published a *PLoS Computational Biology* paper that describes their Spatially Explicit General-Purpose Model of Enteric Tissue (SEGMEnT). The model incorporates existing knowledge of how gut epithelial cells behave and respond to inflammation.

An's team has since harnessed a supercomputing version of this knowledge-based model to characterize the clinical trajectories of individual patients. As reported in March 2015 in *PLoS ONE*, the researchers calibrate the model with data from a clinical trial on patients with pouchitis to see if certain features of their model have predictive power—to determine, for instance, at what point physicians should consider putting patients on antibiotics to hold off development of pouchitis.

An's overarching goal is to develop models that describe how an individual will behave over time and explain how a particular trajectory could be changed. "In medicine it's not sufficient to just prognose and diagnose. We want to be able to control what's going to happen to you," An says. "Models like this can provide that answer." □



Gary An's agent-based models reflect the physical form of the intestinal tissue they are modeling. Panel A shows a histological cross section of ileal tissue (top) and a scanning electron microscopy image of the mucosal surface of ileum (bottom), while panel B shows the topology used in An's model, with crypts and villi represented by a matrix of rectangular prisms. Each individual crypt or villus is then "unwrapped" onto a 2-dimensional grid (Panel C), on which signaling interactions, morphogen diffusion and physical cellular actions take place. Reprinted from C Cockrell, S Christley, G An, Investigation of Inflammation and Tissue Patterning in the Gut Using a Spatially Explicit General-Purpose Model of Enteric Tissue (SEGMEnT), PLoS Comp Biol *doi:10.1371/journal.pcbi.1003507 (2014).*