NewsBytes

T-Rex in the Slow Lane

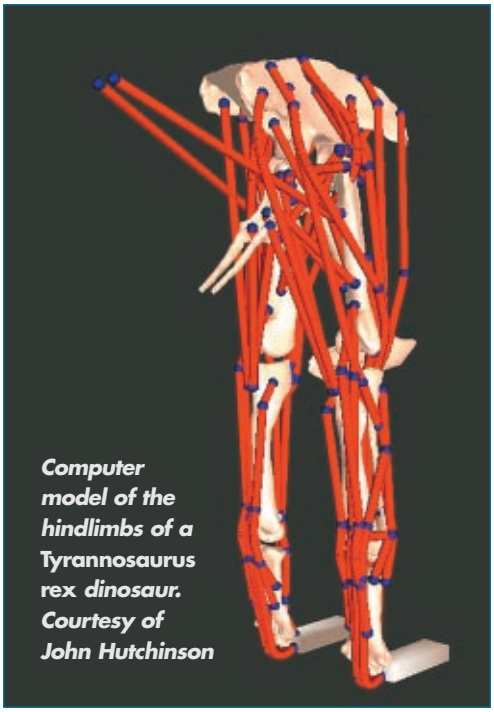
Tyrannosaurus rex is often pictured baring its teeth, crouching, and running swiftly after its prey, but these images are largely based on human fancy. A new computer model of a *T. rex* hind-leg—complete with bones, joints, and muscles—shows that a more upright, slower-moving *T. rex* makes more biomechanical sense. The model is described in the December issue of *Paleobiology*.

"We've brought the biomechanical study of *T. rex* into three dimensions," says lead author **John Hutchinson, PhD**, a lecturer at the Royal Veterinary College in London. "It's the most detailed look at the function of leg muscles in any extinct animal that I've ever seen." Hutchinson created the physics-based simulation in collaboration with Stanford/Simbios researchers **Clay Anderson, PhD**, **Silvia Blemker, PhD** and **Scott Delp, PhD**.

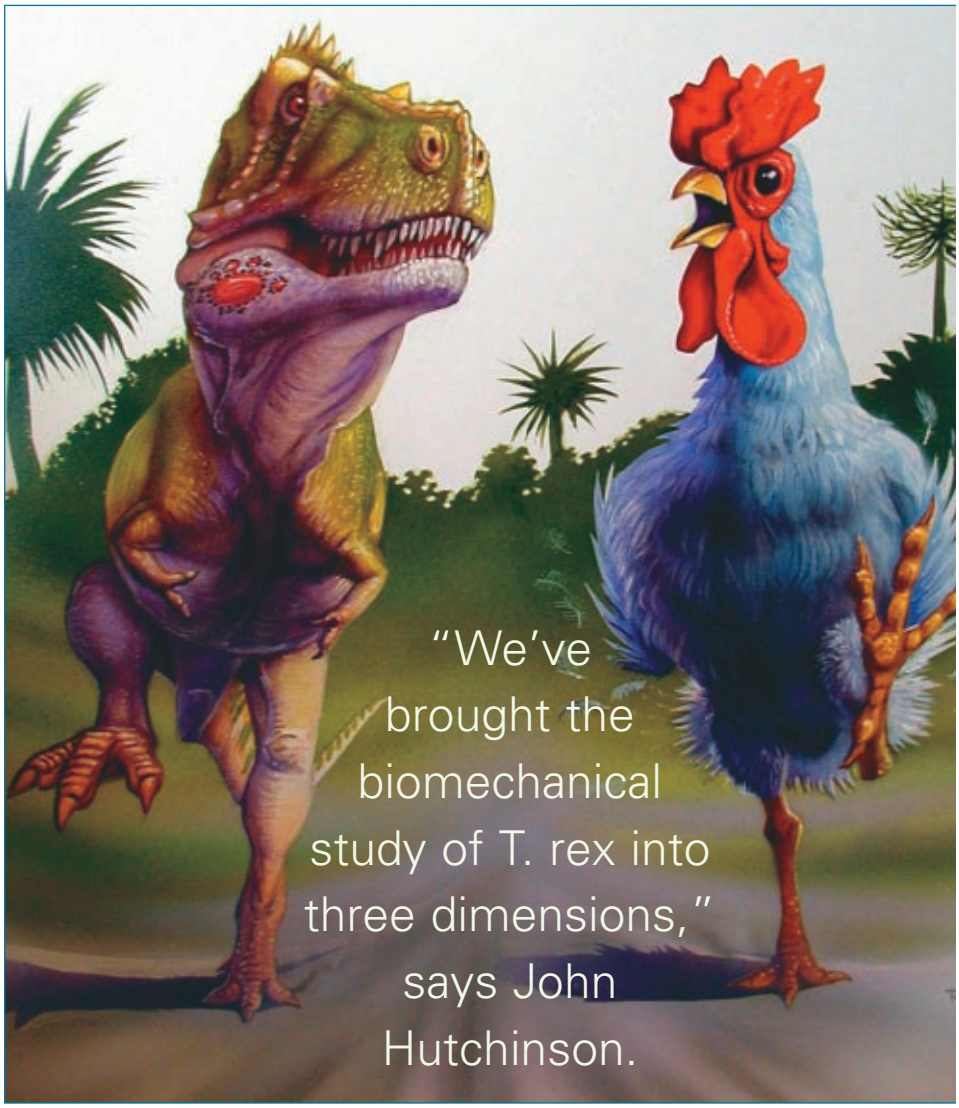
In a landmark 2002 paper in *Nature*, Hutchinson showed that *T. rex* would need giant leg muscles—comprising an impractical 86 percent of its body mass—to support its massive body weight while running at high speeds (45 mph), and he estimated the dinosaur's top speed at 10-25 mph. However, his predictions were based on a simple two-dimensional model and uncertain assumptions about muscle leverage. Muscles with longer moment arms (like longer levers) can support the same body weight using less force and thus can be smaller—so the validity of his conclusions depended on these assumptions.

The aim of his newest model was to directly estimate the moment arms of all the hind-limb muscles. He and his colleagues digitally reconstructed three-dimensional hind-limb bones from an adult *T. rex* skeleton. To the bones, they added hip, knee, and ankle joints, around which they wrapped 37 muscles. Next, they calculated the muscle moment arms during different motions (extension, abduction, etc.) and under varying assumptions for the model parameters.

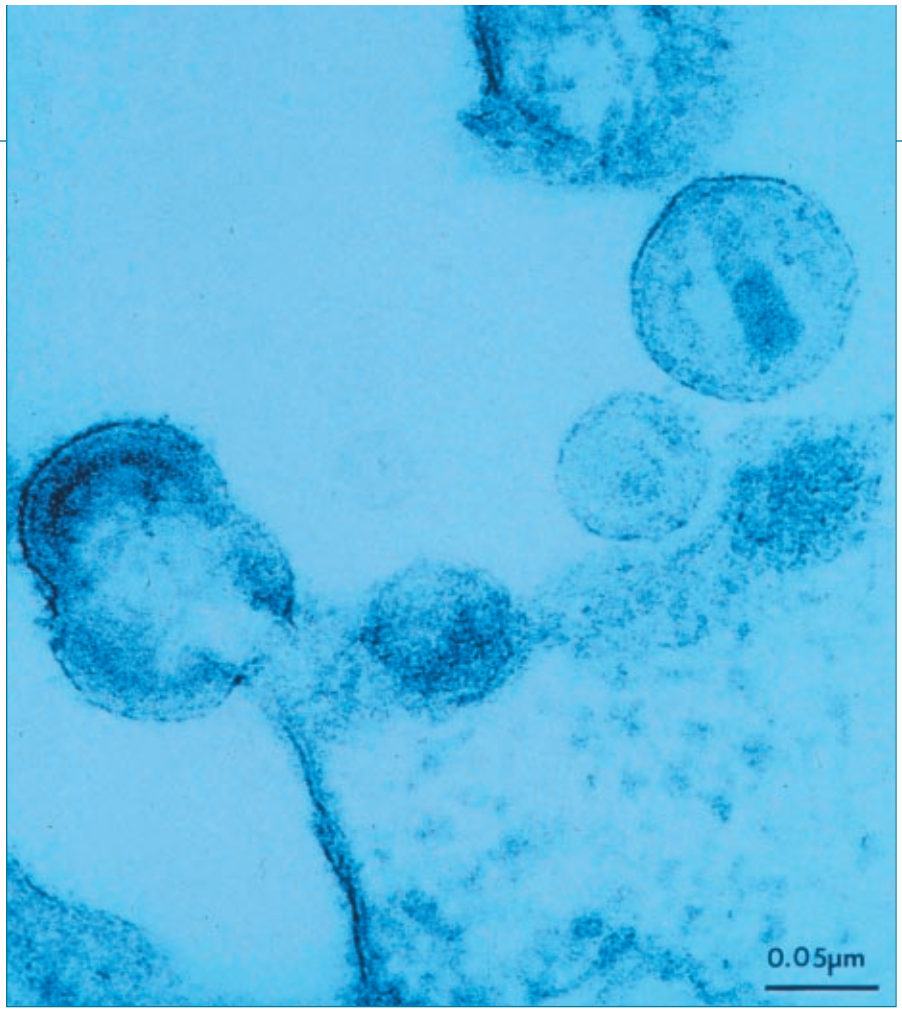
T. rex had more mechanical advantage (longer moment arms) while standing and moving with relatively straight leg joints, rather than in a crouch, Hutchinson says. Surprisingly, the model also showed that Hutchinson had overestimated the creature's muscle leverage and speed in his previous study. "We were actually biasing our analysis to favor a fast-running animal," he says.



Computer model of the hindlimbs of a Tyrannosaurus rex dinosaur. Courtesy of John Hutchinson



An artist's conception illustrating the similarities and differences between a 6,000 kg (about 13,200 lb) tyrannosaur and a chicken. Luis Rey, copyright 2002.



HIV particles budding off an infected CD4 cell. Courtesy of Rolf Kaiser, Institute of Virology, University of Cologne.

The model could be applied to other extinct and extant animals to study how muscle function evolved from very early dinosaurs up to modern-day birds, Hutchinson says. The next step is to add volume to the muscles, which are currently represented just as lines. Eventually, he also wants to make his model move—but only when it is more precise. "It's very seductive to animate a dinosaur moving around, and it makes it seem like there's a lot of science there—but so far it's always just people making stuff up," he says.

"John's work is the first time that a paleobiologist has used sensitivity analysis in reconstructing the locomotive capabilities of an extinct animal like this one," comments **Kevin Padian, PhD**, professor of Integrative Biology at the University of California, Berkeley. In sensitivity analysis, the investigator varies each model parameter across a range of plausible values to see the degree to which a change in each model assumption alters the model output. This quantifies "how wrong you could be," Padian says. He adds: "As a result, his work here and in other papers is really groundbreaking. It sets the standard for future work."

—Kristin Cobb

Pathways to Resistance

Anti-HIV drugs have greatly extended the lives of HIV patients, but treatment is a constant battle to stay ahead of drug resistance. A new model might help guide drug choice by predicting how quickly resistance will emerge under different drug regimens. The model is described in the July issue of the *Journal of Computational Biology*.

"For each drug, there's a set of mutations that are associated with resistance, and those mutations accumulate under drug treatment," says lead study author **Niko Beerenwinkel, PhD**, a post-doctoral researcher in mathematics at the University of California, Berkeley. "We are asking how these mutations develop—in which order and in what time frame."

Beerenwinkel and colleagues adapted an algorithm that was originally designed to study the buildup of mutations that lead to cancer, called a mutagenic tree model. The model finds the most likely order of resistance mutations (represented as a branching tree) and estimates how long it will take to transition from one mutation to the next and finally to full-blown resistance. Their software is publicly available at: http://mtreemix.bioinf.mpi-sb.mpg.de/.

The model looks for statistical patterns in data measured at one time point (cross-sectional data) to infer the order in which mutations occur. For example, if mutation A often appears alone and with mutation B, but B never appears alone, this implies that B follows A. This approach is advantageous since longitudinal data are in short supply, but cross-sectional data are abundant, Beerenwinkel says.

Applying their algorithm to data from the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu/), the researchers reconstructed the known mutational pathways for the first anti-HIV drug, zidovudine (or AZT). They also predicted that dual therapy with AZT plus lamivudine delays drug failure compared with AZT monotherapy, whereas AZT plus didanosine hastens drug failure, due to cross-resistance.

Knowledge gained from this work will help physicians in resource poor areas, by giving them an idea of which mutations a person is likely to carry based on their drug history, comments **Robert Shafer, MD**, who directs the Stanford HIV Drug Resistance Database. Such guesswork is unnecessary in the United States and Europe,

Beerenwinkel and colleagues created a model to predict the most likely order and timing of HIV mutations leading to drug resistance.

where physicians can directly measure the virus's mutational pattern (genotypic testing), as well as its drug susceptibility in the lab (phenotypic testing).

Whether mutagenic trees can add anything to genotypic and phenotypic resistance testing remains to be proven clinically, Shafer emphasizes. "No one has done these models as elegantly as Beerenwinkel has," he says. "But our obligation is to choose systems that work medically, regardless of the elegance of the underlying algorithms."

Responds Beerenwinkel: "While we fully agree that clinical studies are necessary to validate these models, resistance testing can only ascertain resistance that has already developed—we are trying to predict the future."

—Kristin Cobb

Language Lessons

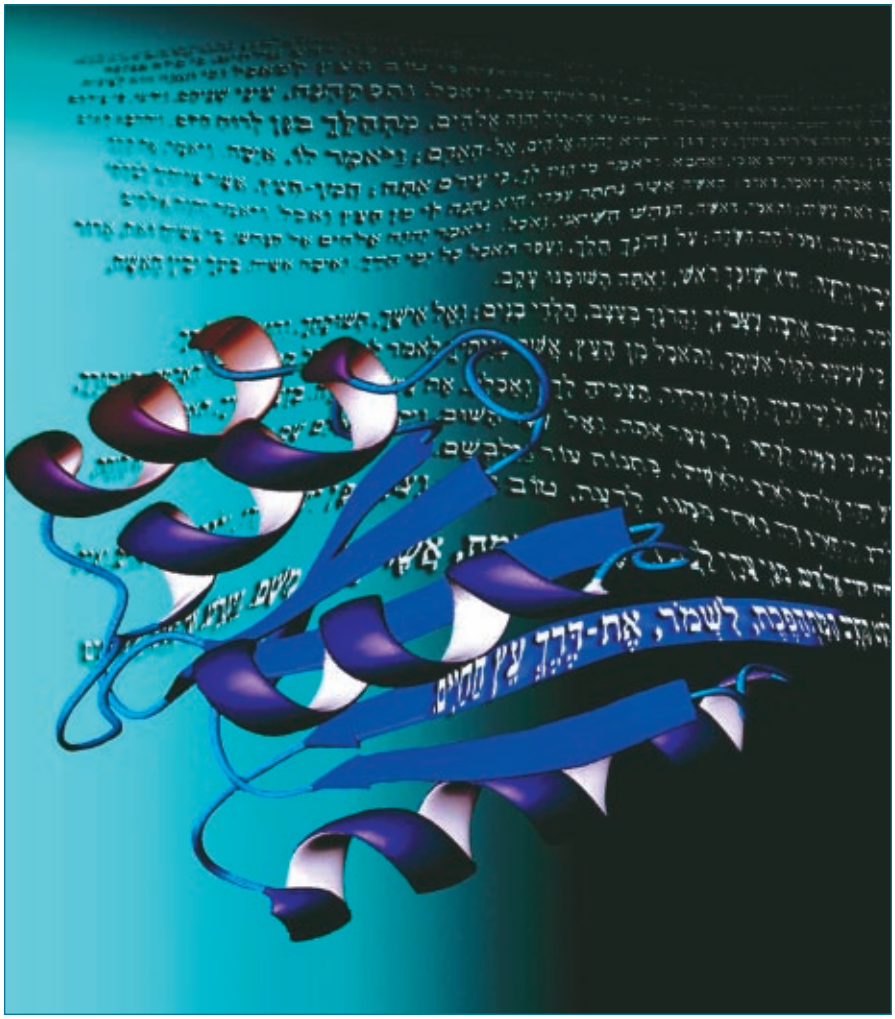
Scientists have devised an algorithm that autonomously learns language—it figures out grammar from statistical patterns in raw text, and generates novel sentences. The algorithm can tease out structure from any string of letters, words, amino acids, nucleotides, or even musical phrases.

"This is the first algorithm to extract grammar in an unsupervised manner from a corpus," says lead author **Zach Solan**, a doctoral student at Tel Aviv University in Israel in **Shimon Edelman**'s lab. The algorithm, Automatic Distillation of Structure (ADIOS), is described in the August 16th issue of the *Proceedings of the National Academy of Sciences*. Whereas most machine-learning approaches to language provide the computer with grammatical rules and words tagged by parts of speech, ADIOS starts with no assumptions.

The program recursively finds recurring patterns and interchangeable sequences, Solan explains. For example, if the original text contains the sentences "I saw a film today," "She saw a film today at the reception," and "Max saw a film today and liked it," then the program extracts the overlap "saw a film today" and adds this phrase to its lexicon. From here, it finds related but non-matching variants, such as "I saw her today," or "I saw him today" and infers that "him", "her", and "a film" are interchangeable in this context (as are: I, she, and Max)—note that interchangeable sets are more general than parts of speech. The program can then create novel sentences, such as: "She saw him today at the reception." and "Max saw her today."

When given a body of 12,700 sentences, ADIOS generated novel sentences indistinguishable (to eight human subjects) in form from the human-generated originals. The program can also accurately evaluate relationships between languages. For

"[ADIOS] is the first algorithm to extract grammar in an unsupervised manner from a corpus," says Zach Solan.



ADIOS proved adept at classifying protein functions by extracting overlapping sequences of amino acids (analogous to overlapping phrases) and grouping the proteins by these motifs. Courtesy: Liat Segal

example, when tested on six translations of the Bible: Chinese, Spanish, French, English, Swedish, and Danish, the program correctly established that Spanish and French are close in origin, and Chinese is far from the others—solely based on structure, not on word roots.

Applied to a bioinformatics context, **Vered Kunik**, a doctoral student at Tel Aviv University, found ADIOS adept at classifying protein functions by extracting overlapping sequences of amino acids (analogous to overlapping phrases) and grouping the proteins by these motifs. Specifically, the algorithm classified 6602 oxidoreductase enzymes from SwissProt database down to their third hierarchical level (Enzyme Commission number 3), and did it better than other known approaches that make assumptions about which motifs yield physical and chemical features such as hydrophobicity, surface tension, and polarity.

ADIOS can also be used in voice recognition and music, Solan says. For example: "You could take the corpus of Mozart and then generalize it to make new melodies."

The project also addresses a long-standing debate in linguistics about whether language acquisition requires innate grammatical ability or whether language can be learned purely from experience.

"It's a proof that the hierarchical structure of language can be learned from the input with minimal assumptions built in," comments Princeton linguist **Adele Goldberg, PhD**. "It's not the only computational model to take steps in that direction, but as far as I know it does go the furthest."

—Kristin Cobb

Red Blood Cell Flexibility Aids O_2 Delivery

A new three-dimensional model of the red blood cell membrane helps explain how the red blood cell squeezes through some of the narrowest channels in the body to deliver its oxygen cargo. The model, unveiled in the October issue of *Annals of Biomedical Engineering*, describes the marvelous flexibility of the red blood cell membrane. Unexpectedly, the model also suggests that such flexibility boosts oxygen diffusion.

The red blood cell is one of the simplest human cells, says **Amy Sung, PhD**, a professor of bioengineering at the University of California, San Diego. Like a small pouch, a thin, flexible protein skeleton just inside the lipid bilayer surrounds a concentrated pack of hemoglobin molecules. Many researchers have attempted to model red blood cells either in two dimensions or by treating them mathematically like sacks of fluid. But these attempts haven't predicted such cells' three-dimensional movements, especially as they deform through narrow capillaries, says **Robert Skelton, PhD**, a professor of mechanical and aerospace engineering at UCSD.

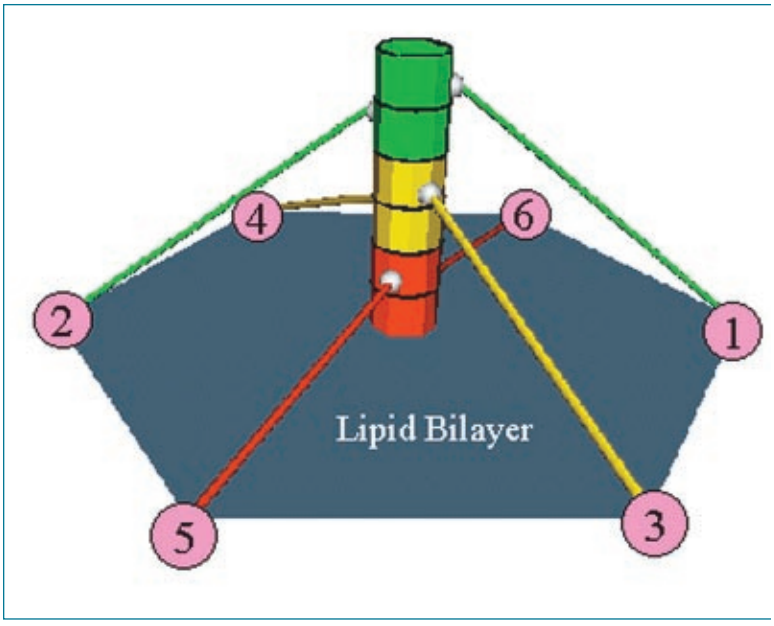
So Skelton and Sung, together with graduate students **Carlos Vera** and **Frederic Bossens**, tried something new. They focused on a core protein complex situated at every junction in the red blood cell skeleton: a short actin protein (called the protofilament) held in place by six radiating spectrin proteins.

The scientists recognized that this complex looks like other man-made and natural structures called "tensegrity" structures. "Tensegrity means getting a stable equilibrium out of sticks and strings by putting the right tension in the strings," Skelton says. Tensegrity often shows up in nanoscale architecture, he adds, in spider fiber proteins or the cellular cytoskeleton.

Sung gave Skelton a three-dimensional map of the coordinates of the red blood cell's protofilament and its radiating proteins. Skelton's lab treated each element either as a stick or a string, generated differential equations to describe the dynamics, and found the equilibrium states of the complex for many hundreds of random initial conditions. Sung's lab simulated likely deformation conditions.

> Modeling the red blood cell skeleton as a tensegrity structure, researchers found that the cell's flexibility boosts oxygen diffusion.

"For the first time," Sung says, "we predicted how the protofilament would be positioned relative to the

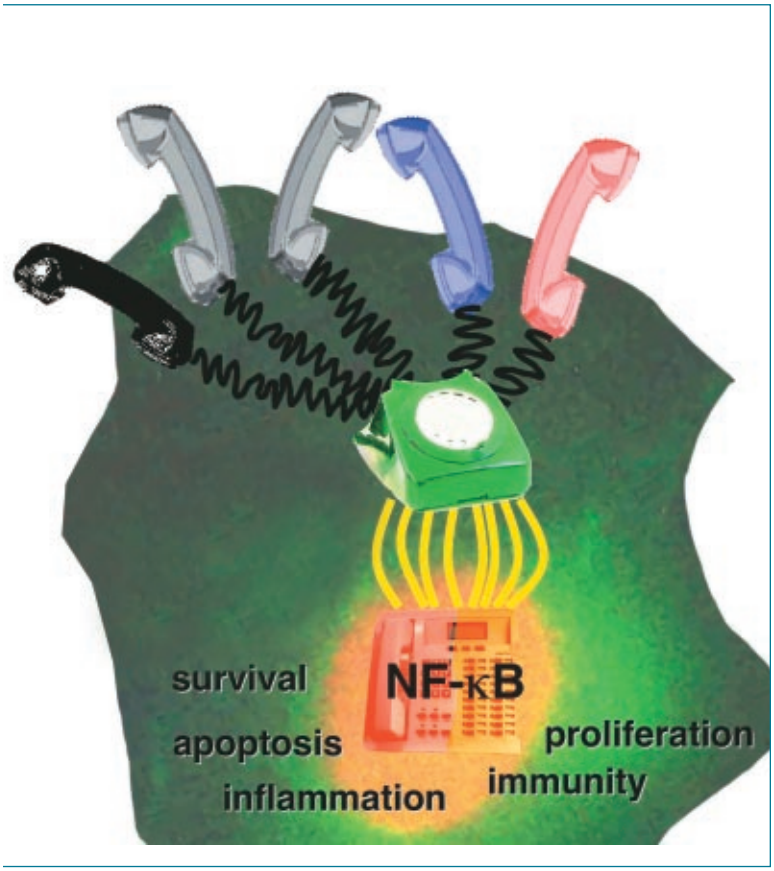


Sung and Skelton modeled a core element of the red blood cell membrane, the protofilament. Their model treats the middle actin protein as a stick and the radiating spectrin proteins as strings. Courtesy of Amy Sung.

other parts of the network and predicted the tension of every single spectrin molecule associated with the protofilament." Not only that, the model predicts that even a slight deformation of the complex causes the protofilament to swing in a wide arc over the surface of the lipid bilayer, enhancing the diffusion of oxygen into the red blood cell in the lungs or out of the cell in the body.

Donald Ingber, PhD, MD, a professor of pathology at Harvard Medical School, says that Sung and Skelton's work "shows the power of modeling cellular microstructure in the way it is really constructed—as a tensed discrete network structure composed of nanoscale elements." This approach, he adds, will not only improve our understanding of the circulatory system, but also help nanotechnologists build biologically-inspired materials for future biomedical, industrial, aerospace and military applications.

—Louisa Dalton



Like a telephone wire that conveys all sorts of messages, the internal cell signaling pathway NF-κB transmits a variety of different signals from the cell surface to genes in the nucleus. Courtesy of Alexander Hoffmann.

Simulated Cell Chatter

In every cell, individual signaling pathways carry diverse messages from surface receptors to genes in the nucleus. Researchers have long wondered how one pathway can keep multiple signals straight. Now scientists at the University of California, San Diego have at least one answer: the message is encoded in how signaling molecules transmit stimuli over time. Their work was published in *Science* on September 16, 2005.

"If you take the analogy of pathways as being electrical wires that connect the receptors with the genes, then these wires are not just wires that you turn on and off like a light switch," says **Alexander Hoffmann, PhD**, assistant professor of chemistry and biochemistry at University of California, San Diego. "They are more like telephone wires—wires that transmit an enormous diversity of different words."

Most laboratory experiments involving cell signaling portray a static image of the cell, not a dynamic one, says Hoffmann. Typically, researchers bombard a pathway with a protein to see if it turns a signaling pathway on or off. Such a method helps link a protein with a pathway, but precludes the possibility of understanding how multiple signals are transmitted simultaneously or through time.

So when Hoffmann and UCSD graduate students **Shannon Werner** and **Derren Barken** set out to study the NF-κB signaling pathway, they decided to build a dynamic computational model. The NF-κB pathway is involved in immunity, cell development, and inflammation, and—when it goes awry—in cancer and arthritis. The researchers assigned a differential equation to each protein interaction in the pathway, then refined and adjusted the model by comparing model simulations to cell assays.

Once the computational model matched the lab experiments, the researchers took the model further than the experiments could go. They used it to compare messages from two incoming stimuli: one that strongly triggers inflammatory genes and another that activates survival genes. They discovered that the first message set off pathway activity that waxed with time while the other one triggered activity that waned. It is this difference in activity over time that tells the cell which genes to activate. Thus, says Hoffmann, different stimuli elicit the same pathway, but with different temporal dynamics.

Researchers have long wondered how one pathway can keep multiple signals straight.

It is too early to tell whether other pathways work the same way as NF-κB, remarks **Douglas Kell, PhD**, professor of chemistry at the University of Manchester. He adds, however, that modeling is important because "frequency-encoding of signals may be a means of effecting specificity." Hoffmann adds that "more and more studies in biology will require computational modeling in order to make sense of what is going on."

—Louisa Dalton □