Starting with a rectangular prism filled with material, as well as boundary conditions that fix the height, the algorithm iteratively designs the optimal shape to withstand the loads (the direction and size of the forces) exerted by, say, chewing and swallowing. Videos accompanying the research show the gradual evolution of the optimal shape. "The algorithm is such that it will take you to the correct shape in less than an hour," Sutradhar says.

Sutradhar's group is now testing the optimized shape in a skeletal model, confirming that it does actually withstand the required forces. In the future, scientists might be able use tissue engineering to grow bones on a topologically optimized scaffold, he adds.

Nationwide Optimization of Live Kidney Donor/Recipient Matches

People who need kidney transplants often have friends and relatives who are willing to donate. They will even donate to a different person as long as their loved one gets a kidney out of the transaction. This might happen either in a "cycle" of multiple donor/recipient pairs (pair A gives to pair B who gives to pair C who gives to pair A) or in a chain (where an altruistic donor sets off a chain reaction of donation from one pair to the next and the next and so on).

Organ centers are therefore faced with the constant problem of matching multiple potential donors with multiple potential recipients. This scenario created a challenging computational problem that caught the attention of **Tuomas Sandholm**, **PhD**, professor of computer science at Carnegie Mellon University. He created an optimization algorithm that, following a successful pilot program, began being implemented nationwide in October 2010.

Unlike predecessors who have tackled the problem, Sandholm's algorithm solves the problem optimally and scales to larger populations without any simplifications. All the possible combinations of cycles would be "more than the number of atoms in the universe," Sandoholm says. "The algorithm has to prove that there are combinations that aren't worth trying. Otherwise you're dead in the water." His algorithm identifies combinations of cycles and chains that you shouldn't even try. The problem has interesting computational bits and pieces, Sandholm says. For example, he had to constrain the algorithm such that no donor gives out more than one kidney; assign weights to maximize better quality over lower quality matches; deal with the fact that computer memory was the bottleneck rather than speed; and design the rules of the exchange.

Optimizing X

The work described here suggests additional opportunities: optimizing drug protocols for hepatitis; optimizing hip replacement designs; and optimizing liver and bone marrow transplants. And there may be other low-hanging fruit out there just waiting for someone to pluck them and find amazing satisfaction. As Sandholm says, "It's very rewarding and unusual for a computer scientist to be able to save lives like this." □

LIFE IS CROWDED: Modeling the Cell's Interior

By Kristin Sainani, PhD

Molecules in cells behave like people in crowded subway cars. Because they can barely budge or stretch out without bumping into a neighbor, they move more slowly, smush themselves into more compact forms, and coalesce into aggregates more often than in a less congested setting, says **Allen Minton**, **PhD**, a physical chemist at National Institute of Diabetes and Digestive and Kidney Diseases, who coined the term "macromolecular crowding" in 1981. In addition, short distances separate crowded molecules, so they may also exert forces on one another, sometimes altering the effects of limited space.

In the past, intracellular crowding was routinely ignored in both experiments (which are typically run in uncrowded solutions) and computer models. As a result, scientists' understanding of intracellular biology might be inaccurate. But in studies during the past five or six years experimentalists have added crowding agents—complex polysaccharides that take up space—to their test tubes to get a better picture of crowding effects. And modelers are using recent gains in computational power to consider the complex interactions of hundreds or thousands of macromolecules at once. 2010 saw these computer models begin to yield surprising insights about molecular diffusion as well as protein folding and function.

> ...Modelers are using recent gains in computational power to consider the complex interactions of hundreds or thousands of macromolecules at once.

Slow going: Modeling diffusion

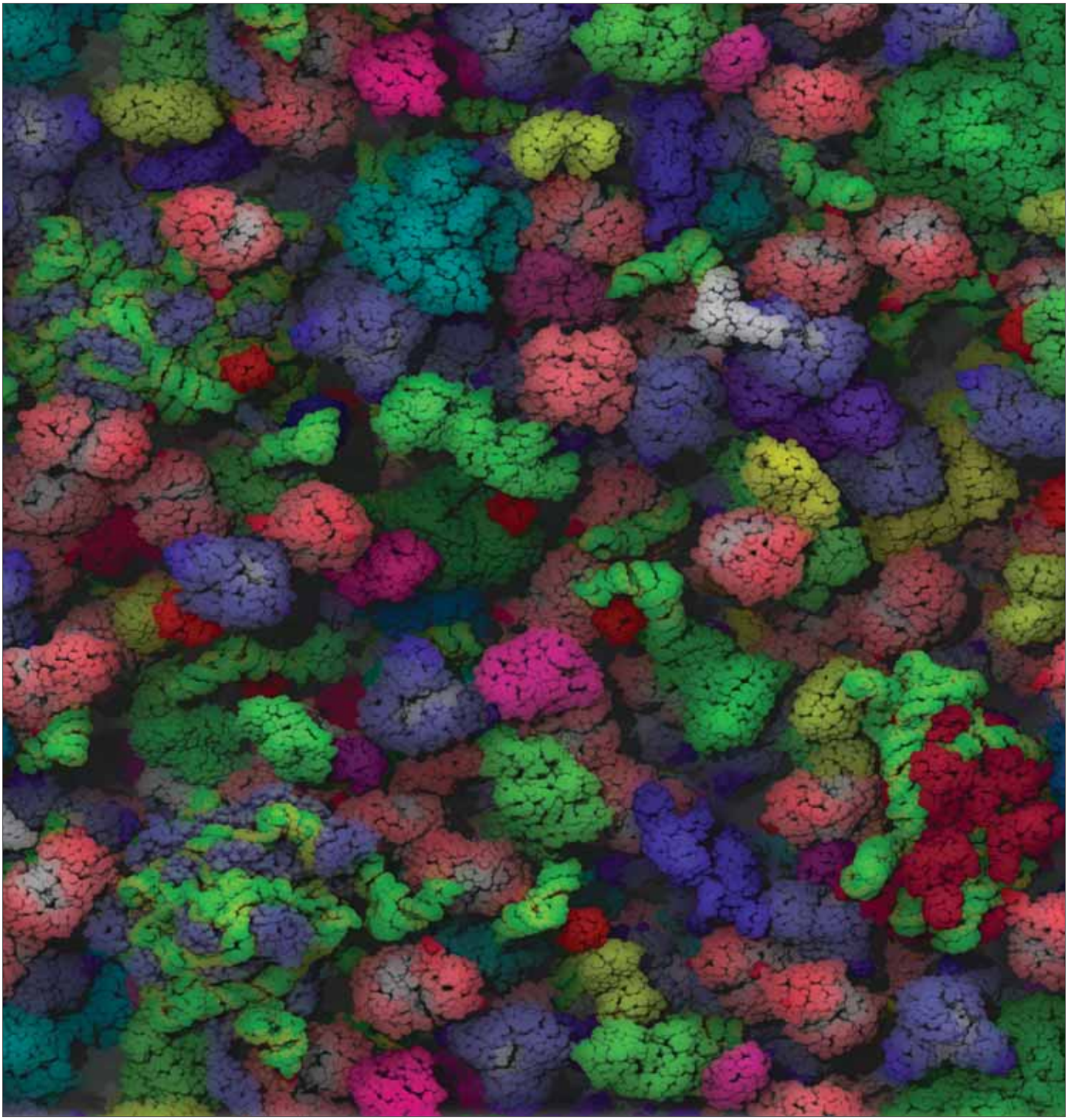
Fluorescent-tagged proteins move 10 to 15 times more slowly inside an *E. coli* bacterium than in a test tube, says **Jeffrey Skolnick**, **PhD**, professor of systems biol-

ogy and director of the Center for the Study of Systems Biology at Georgia Tech. To try to work out the exact causes of this slow down, his team ran Brownian dynamics simulations of a virtual *E. coli* packed with more than 1000 macromolecules (including 15 unique types). They reported their results in October 2010 in the *Proceedings of the National Academy of Sciences*.

Crowding alone—just molecules taking up space, or "excluding volume"—explained only about one third of the reduction in diffusion speed. But the combination of excluded volume plus hydrodynamic interactions—molecules creating wakes like sailboats in a lake—achieved the 10 to 15 percent reduction.

"If you have a whole bunch of sailboats, your behavior is going to be modified by the presence of the wakes created by all the other sailboats," Skolnick says. "In the same way, when one molecule starts to move it creates an eddy in the

***The Crowded Cell:** This picture shows an atomically detailed model of the crowded* **E. coli** *cytoplasm, including the 50 most abundant macromolecules. RNA is shown as green and yellow. Reprinted from: McGuffee SR, Elcock AH (2010) Diffusion, Crowding & Protein Stability in a Dynamic Molecular Model of the Bacterial Cytoplasm.* **PLoS Comput Biol** *6(3): e1000694.*



solvent which perturbs the flow around other molecules."

Hydrodynamic interactions had largely been ignored in previous cell simulations, because they act over a long range and time frame and thus are computationally expensive to implement. "I'd rather throw it away if I could," Skolnick says. His team had to reduce the total number of molecules in the simulation to about 400 to keep it computationally tractable.

Skolnick's team also considered weak attractive interactions, such as van der Waals forces. If you make proteins sufficiently "sticky," you can slow diffusion to any speed—even zero, Skolnick says. But his team showed that these forces are much more dependent on particle size, when stickiness dominates, as compared to hydrodynamic interactions. "So it seems that crowding and hydrodynamic interactions are the dominant effects," Skolnick says.

Squished together: Modeling protein folding and function

When space is at a premium, proteins are driven to fold and compact. But accounting for crowding in simulations of protein folding takes enormous computing power.

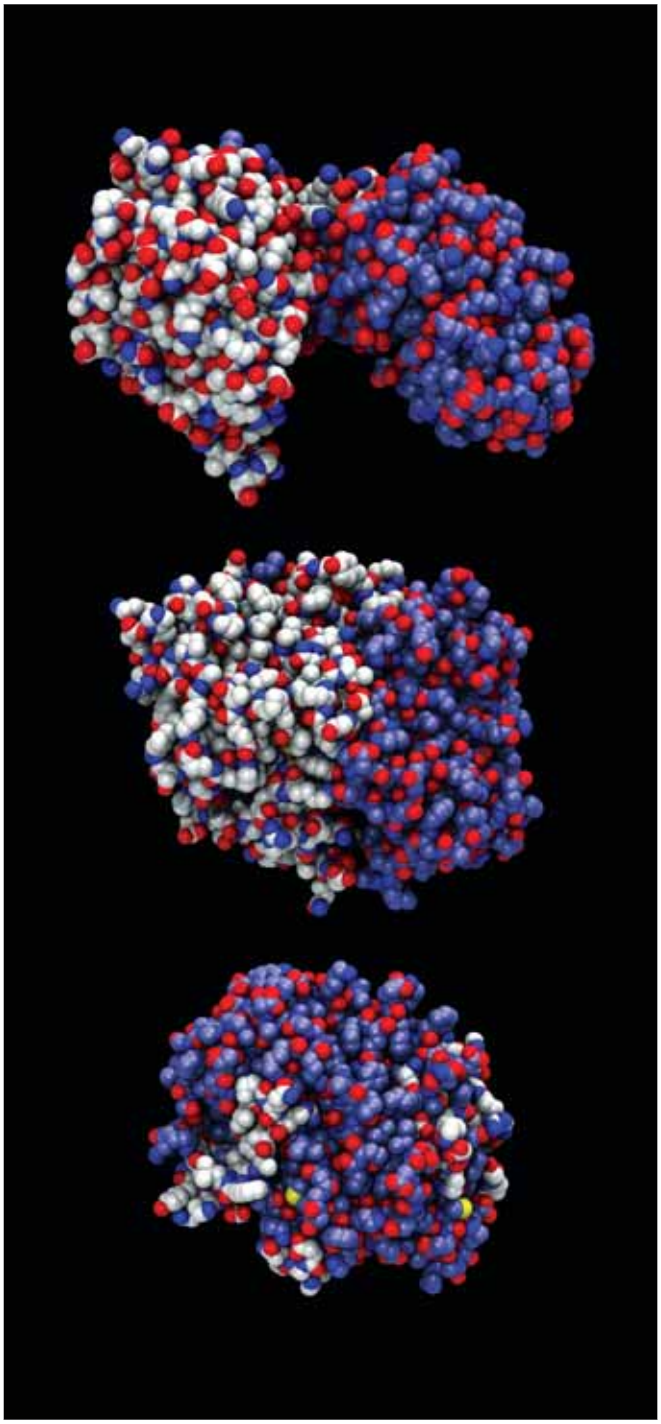
"It's a very intimidating task to think about not only just one protein, but many, many proteins," says **Margaret Shun Cheung, PhD**, assistant professor of physics at the University of Texas, Houston. In 2005, she and her mentor—**Devarajan Thirumalai, PhD**, professor of chemistry and biochemistry at the University of Maryland—published some of the first simulations of protein folding in crowded conditions.

In an October 2010 paper in *PNAS*, Cheung and her team reported the effects of crowding on PGK, an enzyme involved in glycolysis (the breakdown of sugar). In its native state, PGK is shaped like PacMan—it has two subunits where substrates bind, connected by an open hinge. Researchers thought that substrate binding caused PacMan to close his jaws, bringing the substrates together and igniting the reaction.

But using coarse-grained models, Cheung found that the enzyme actually remains in a closed, non-native state in the crowded cell (see video at: http://vimeo.com/15969373). Cheung's experimental collaborators attached fluorescent tags to PGK's two subunits and confirmed the finding.

The closed conformation keeps the binding sites near each other, allowing the substrates to bind one another quickly. PGK can therefore act 15 times faster *in vivo* than in dilute solution. "This indicates that protein function inside a cell may be very different than in a test tube," Cheung says.

Cheung, like many others, models crowding agents as simple spheres, to save computing power. But such models may miss important protein-macromolecule interactions, says **Adrian Elcock, PhD**, associate professor of biochemistry at the University of Iowa.



***Protein Compactor:** The crowded conditions in a cell cause the enzyme PGK to compact. The protein folds into three states depending on the level of crowding—from a more open conformation (top) to the most closed conformation (bottom). Courtesy of: Margaret Shun Cheung, University of Texas, Houston.*

In a March 2010 paper in *PLoS Computational Biology*, his team described an atomically detailed model of *E. coli* cytoplasm, including about 1000 instances of the 50 most abundant macromolecules. The molecules were modeled as "hollowed out" rigid shells, with atomic details only on the surface. It took a year to run the simulations.

Crowding is expected to stabilize protein folding. But when Elcock's team considered the thermodynamics of two particular *E. coli* proteins, they found that folding was actually less stable *in vivo* than *in vitro*. The reason: electrostatic and hydrophobic forces actually countered the excluded volume effects.

So just as Skolnick's work showed the importance of hydrodynamic interactions in a crowded environment, their work showed the importance of electrostatic and hydrophobic interactions.

"I think both studies are first generation models. Second generation models will have to take aspects of both," Elcock says.

The Future: Modeling the cell and beyond

Understanding the effects of crowding on macromolecules is a necessary first step toward whole cell simulation, Skolnick concludes. "And now given the algorithms and the computational resources, it's not a preposterous question to begin to look at these things." □