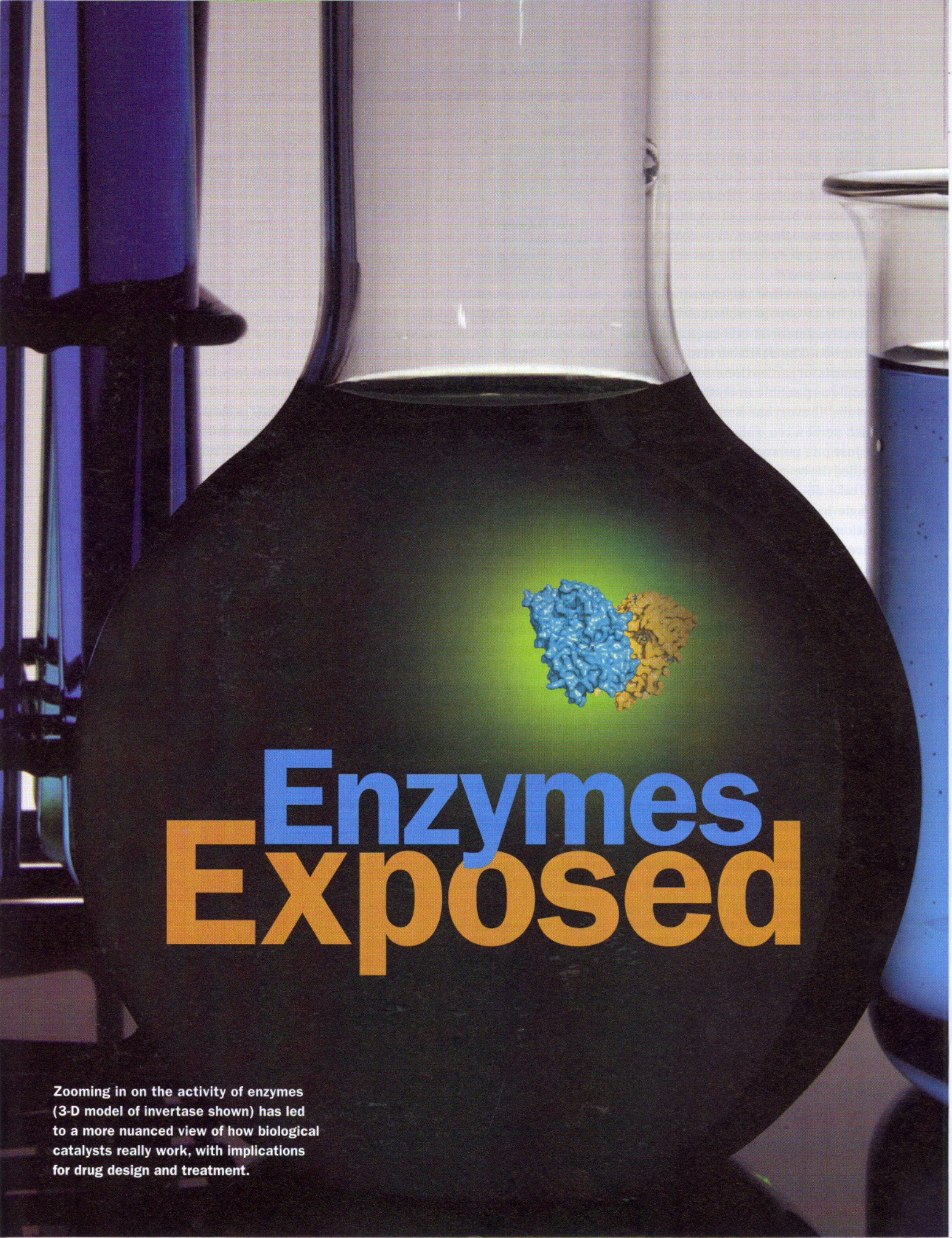

Enzymes Exposed

Zooming in on the activity of enzymes (3-D model of invertase shown) has led to a more nuanced view of how biological catalysts really work, with implications for drug design and treatment.

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Clearer views of the cell's movers and shakers threaten a century-old mainstay of biology

By Lisa Grossman

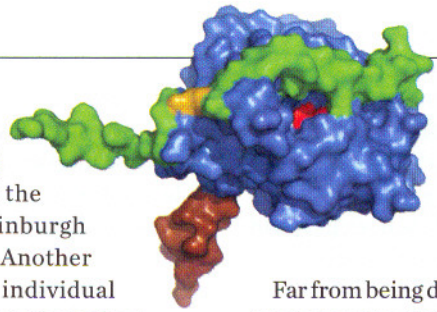
In some ways, cells are a lot like cities. Maps of a cell's innards depict thoroughfares linking factories that build large molecules to post offices where those molecules are packaged up and shipped out, for example. The cell's denizens — proteins and other molecules — shuttle around busy cellular byways like people on the street, meeting up, interacting and keeping the whole enterprise going.

But anyone who has ever been delayed on the way to an important meeting knows something about cities that biochemists are just beginning to learn about cells: Maps don't capture a lot of details — traffic, closed roads, a downed tree — that can drastically slow a journey.

For almost a century, biologists trying to describe cells' inner workings have assumed that the differences between map and street didn't matter. That has been especially true for studies of the cell's go-to, workhorse proteins called enzymes, which orchestrate the majority of the chemical reactions necessary for life. A revered textbook formula that describes how these crucial molecular catalysts speed up reactions, the Michaelis-Menten equation, assumes that enzymes don't usually get stuck in traffic. Enzymes are supposed to meet other molecules at regular intervals and do their transactions at a constant speed, more like workers on an assembly line than urban pedestrians.

In most laboratory experiments, researchers make sure that molecules can move freely and interact often, so the classic formula seems to work well. But lab experiments don't reflect the inner lives of cells. And the differences could dethrone the venerable equation.

Because of the crowds, in a cell it's more difficult for enzymes to find their partner molecules than it is in a test tube, biophysicist Ramon Grima of the University of Edinburgh recently showed. Another study noted that individual cells can have different numbers of enzymes — even when the cells are otherwise identical. Combined, the new results could mean models of enzyme kinetics based on the Michaelis-Menten equation are wrong.



Shape-shifting enzymes like CalB are revealing the role of form in reaction speeds.

"It's a system that people thought they had understood for 100 years," says enzymologist Kerstin Blank of Radboud University in Nijmegen, the Netherlands. "Now we get some new information that, a little bit, turns everything upside down."

When scientists model the chemistry in a cell, Michaelis-Menten is the default equation for enzymes. "It has a broad impact," Grima says. "Given any biochemical pathway, you'll always find that at the backbone of the pathway you will have a few enzymes. When you're modeling that enzyme, you will naturally assume a Michaelis-Menten equation for it."

By zooming in to the street view, scientists hope that they can draw a more accurate map of the cellular city. Experimental methods for watching enzymes in cells aren't yet good enough to see how important variations in these chemical reaction speeds actually are. But if it turns out that the Michaelis-Menten equation doesn't accurately predict how fast enzymes work in living cells, it could change everything from introductory biochemistry classes to strategies for cancer treatments.

Far from being discouraged about having to rewrite their textbooks, though, scientists are now dreaming about how to use this newfound knowledge to engineer new drugs or biofuels. "Ultimately," says Nathan Price of the University of Illinois at Urbana-Champaign, "you want to understand those processes so you can control them."

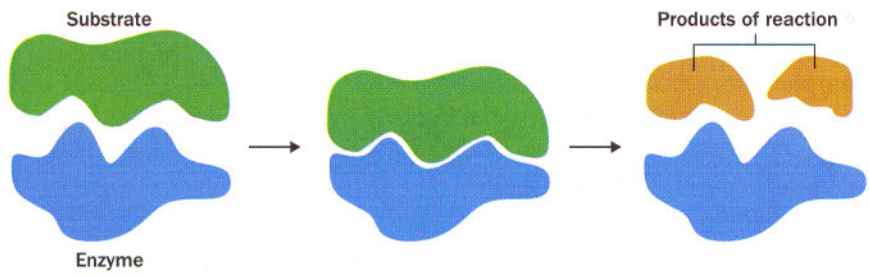
Shape-shifting enzymes

Enzymes make the cellular city run on time. Reactions that would take more than 300 years unassisted can take about a second when an enzyme steps in. By embracing a specific partner molecule, called the substrate, and morphing it into something new, enzymes enable everything from transcribing DNA to digesting food to generating light in fireflies. So understanding how enzymes work is crucial for understanding how cells work — and for manipulating them.

When Leonor Michaelis and Maud Menten published their now-famous paper in *Biochemische Zeitschrift* in 1913, watching an individual enzyme at work was impossible. To figure out how quickly enzymes help transform neighboring molecules from one form to another, the duo had to make do with analyzing test tubes full of billions of molecules.

Michaelis and Menten focused on the enzyme invertase, which helps break down sucrose, ordinary table sugar. If

Lowering the energy Enzymes make reactions in the body go, and go fast, by permitting them to proceed with less energy input than would otherwise be needed. The substrate molecule is held by weak forces, such as hydrogen bonds, in a suitably shaped active pocket in the enzyme. This facilitates the conversion of substrate into products, which are then released without using up the enzyme.



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they could have somehow seen exactly what was happening in their test tubes, Michaelis and Menten would have seen the enzyme embrace a sugar molecule (fitting part of it neatly into a cleft) and then breaking it in two. The resulting simple sugars, fructose and glucose, go on to become energy sources for the cell, and the enzyme sits and waits for a new sucrose molecule to come around.

Michaelis and Menten found that the time it takes to transform a spoonful of sucrose to glucose and fructose depends on how much sucrose there was to begin with. The more sucrose, they showed, the faster the reaction — up to a point. After that, the reaction went at a steady pace.

Biologists explained this phenomenon by picturing enzymes and their partners fitting together like a lock and key. Each type of enzyme generally works with only one type of partner, and the two shape themselves to fit together perfectly. But each enzyme can couple with only one partner at a time. When all the enzymes are busy, new partners have to wait for an enzyme to free up.

Researchers were more or less satisfied with that picture for the next 85 years, and plugged in the Michaelis-Menten formula to determine reaction rates in cells. As far as most lab experiments went, it worked.

But in 1998, Sunney Xie, now of Harvard University, and colleagues used a fluorescent marker to watch a single molecule of the enzyme cholesterol oxidase as it met and morphed its partners one at a time. The researchers noticed something strange: The enzyme didn't always work at the same speed.

"If you had simple chemical reactions, you'd expect these times [between one reaction and the next] to be constant," Blank says of Xie's work. "These times are not constant."

The speeds didn't vary randomly, either. The enzyme seemed to work quickly for several partner molecules in a row, slow down for the next several molecules, then speed up again. If one reaction took a particularly short time, the next one was more likely to go quickly as well, as if the enzyme could remember how long it spent on the last reaction it performed.

In 1998 in *Science*, Xie proposed that the enzyme was flip-flopping between many different shapes, each of which did the same job at a different speed.

"For many years we just thought that the substrate fits in the enzyme with this lock-and-key mechanism. That's what we all learned at school," Blank says. "It's basically not true."

> "We just thought that the substrate fits in the enzyme with this lock-and-key mechanism.... It's basically not true."
>
> KERSTIN BLANK

The leading hypothesis posits that one shape fits best with the partner molecule and so works more efficiently, but takes more energy to maintain, Blank says. Other shapes may not work as well, but are more energetically relaxed. Scientists think that the enzyme chugs along in a high-energy shape for as long as it can, but inevitably slouches into a couch-potato shape. It stays slouched until a new burst of energy, perhaps from temperature changes in its environment or from random fluctuations, kicks it back into high-efficiency mode.

"The enzyme molecule, like us, works hard for a while and then slows down," Xie says.

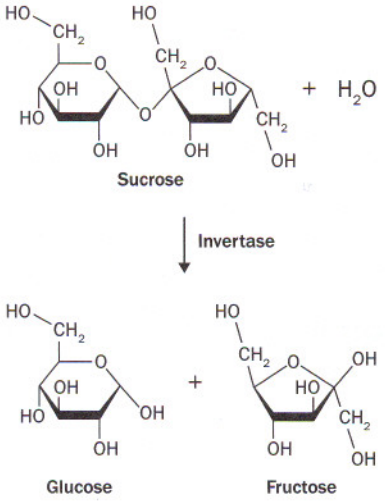
Microscopes still aren't sensitive enough to take snapshots of these shape-shifting enzymes in action, but a decade of research backs up Xie's idea. In one particularly illustrative case, Blank and her colleagues recently found that when they tug on part of the enzyme CalB using an atomic force microscope, the enzyme works faster. Pulling the enzyme may open it up, like pulling on a tab in a pop-up book, changing the enzyme's shape and ability to catalyze reactions.

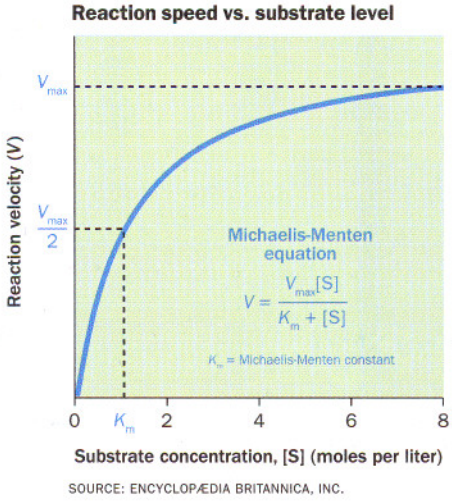
The ease with which the body absorbs medication and digests food may depend on how much time an enzyme spends in each shape. Blank suggests that shape-shifting enzymes could even drive evolution, if a genetic mutation were to enable a helpful enzyme to stay in a more efficient shape for a longer time.

These shifting reaction rates should shift the outcome of the Michaelis-Menten equation, too. Initially, the scientific community reacted with confusion: If Michaelis-Menten was wrong, why had all the experiments so far worked?

"When Sunney began these researches in the late '90s, people said, 'Gee, if you have these fluctuating rates, how come we almost always see Michaelis-Menten

Sweet biochemistry Leonor Michaelis and Maud Menten's classic work on the speed of enzymatic reactions was based on studies of the conversion of table sugar, or sucrose, into two simpler sugars, glucose and fructose, by the invertase enzyme (left). Michaelis and Menten found that the reaction rate rises with the concentration of sucrose before leveling off at a maximum, shown in graph (right). These studies led to their long-used equation for determining reaction rates.





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kinetics? Something must be wrong with your experiment,'" recalls Attila Szabo of the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Md.

In 2005, Xie resolved the paradox. He and his colleagues found a partner molecule that let off a burst of fluorescence after reacting with an enzyme. The researchers watched the molecular fireworks show for roughly 20,000 reaction cycles, about 40 times more reactions than were captured in the 1998 studies.

The enzyme was still wiggling and shifting its efficiency every few reactions, the researchers found. But given enough reaction cycles, the differences averaged out. "Enzymes seem to have a changing personality," Xie says. "But in spite of that, the Michaelis-Menten equation still holds."

The biochemistry community seemed to breathe a sigh of relief. The 2006 issue of *Nature Chemical Biology* where Xie's paper appeared also included a commentary titled, "Michaelis-Menten is dead; long live Michaelis-Menten!"

Back-alley trysts

Yet, as Xie and others predicted, still another challenge to the classic equation has been brewing. Recent studies of individual cells suggest that while some of the basic assumptions behind Michaelis-Menten may work in the lab, they don't always work for real cells.

"In test tubes, you have a very artificial environment," says Grima, who has explored the basic question of how reactions actually happen in cells.

Cells have a few obvious differences from test tubes. For one thing, cells are crowded. Just the largest molecules inside take up between 5 and 40 percent of the physical volume of a cell. What free space remains is found in tiny compartments that range from about 50 nanometers to just a few micrometers on a side. Enzymes themselves may be between a few and a hundred nanometers long. Some enzyme-assisted reactions can take place only inside the nucleus or other cellular organelles. Inside real cells, liaisons between enzymes and their partners may be relegated to the back alleys, where only a few individual molecules can fit at a time.

This means that it's not always easy for enzymes and their partners to find each other. Biologists have shown that cells have what are called active transport networks, filaments that molecules can slide along to travel between meeting places. If enzymes can't meet locally, they have to take public transport.

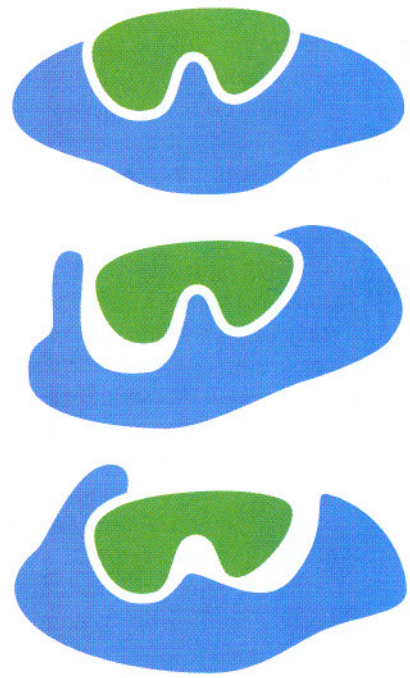
If reactions inside cells are like back-alley trysts, reactions inside test tubes are like square dances in a big hall. With such a large space to move around and researchers constantly mixing the solution, every enzyme is almost guaranteed to dance with every potential partner.

These differences ought to influence how quickly enzyme-aided reactions go, Grima reasoned. There should be some big departures from the Michaelis-Menten equation inside real cells.

In 2009, Grima used mathematical models and computer simulations to show that two basic assumptions behind the Michaelis-Menten equation throw its predictions off in real cells. First, he considered the number of molecules interacting. In a test tube, billions of molecules could come together. But in a cell, only 10 to 100 may meet at any given time.

Accounting for this and other "noise" in a cell, Grima's model suggests that enzyme reactions in real cells proceed as much as 20 percent slower than Michaelis-Menten predicts. Next, he considered active transport. If partners must ride intracellular subway lines to meet up with their enzymes, Grima found, Michaelis-Menten may overshoot the real reaction rate in a cell by as much as several hundred percent.

A faster reaction rate translates into more reaction products from the same amount of enzyme. For drug designers, miscalculating the amount of product throws off the prediction of how much enzyme should be added to begin with. To explore such implications, Grima ran his simulations for a made-up drug that works by binding to an enzyme before the enzyme's proper partner can reach it, a phenomenon called enzyme inhibition. In the case Grima studied, the amount of the drug needed to effectively combat the theoretical disease was seven times higher than the amount predicted by Michaelis-Menten.



Flip-flopping forms As an enzyme (blue) shifts its shape, its fit with a substrate molecule (green) shifts too, changing the enzyme's ability to drive a reaction. Frequent changes in form translate into more variability in reaction rate than the Michaelis-Menten equation takes into account. But the average reaction rate, it turns out, is in line with the equation.

"When I computed those estimates for drug dosages, that's when I had the 'aha!' moment," Grima says. "That's when I thought, oh wow, these things may be actually important."

Population effects

Price and Pan-Jun Kim of the University of Illinois at Urbana-Champaign think their results, like Grima's, could have important implications for drug development. Even if Michaelis-Menten does work for one particular cell, variations between cells can pose another threat to the equation — and to the efficacy of drugs designed using it.

"Any enzyme in a chemical soup has a potential chance to catalyze substrates anywhere else in the chemical

T. DUBÉ

soup," Kim says. This was the case in Xie's single-enzyme studies: Thousands of partner molecules floated past a solitary enzyme, and each one had an equal chance of reacting with it. But in real cells, each enzyme usually meets only with the partners that happen to live with it.

And different cells may make different numbers of any particular enzyme, even when the cells are genetically identical. A 2008 study in *Science* showed that this difference can literally mean life or death for a cell: Tumor cells that survived treatment with chemotherapy were shown to make more molecules of a particular enzyme than cells that succumbed to the drug, hinting that the enzyme might play a role in drug resistance.

This variation could also mean that even if one cell follows the Michaelis-Menten equation, large groups of cells taken all together might not, Kim says. And drugs designed using equations that ignore the differences between cells could therefore be less effective.

"Even in an ideal situation where the Michaelis-Menten equation might be working well inside a single cell, it is still unavoidable to witness its breakdown at a population of cells," Kim says.

Kim and Price showed mathematically that using the Michaelis-Menten equation to calculate how fast a large group of cells will perform a reaction gives a different answer than averaging the reaction speeds of each individual cell. By comparing the old equation with new data on single cells, the researchers found that the standard predictions for how fast enzymes work can be off by about 25 percent.

"When we first started this we thought, oh, this looks interesting, but maybe it's negligible. Turns out they have pretty huge effects," Price says. "For any scenario where we know protein copy number varies between cells, which looks to be common, you'd be off."

Enzyme engineering

Understanding how the differences within and among cells change reaction rates can also eventually let scientists engineer better enzymes. Most of Price's research focuses on building computational models of metabolic networks in cells, which means that he is concerned with what the cell eats and excretes. Ultimately, he says, better models will mean better control.

"You could have an organism that eats something toxic and spits out a biofuel, or something like this," Price says. "You could convert compounds that are either cheap and abundant, or deleterious, and make them into something valuable and useful and good for the environment."

Not everyone is convinced that the Michaelis-Menten equation is really doomed, however. When Grima presents his results at conferences, "a lot of people get enthusiastic, and a lot get defensive."

A major problem is that the new models don't have experimental data backing them up.

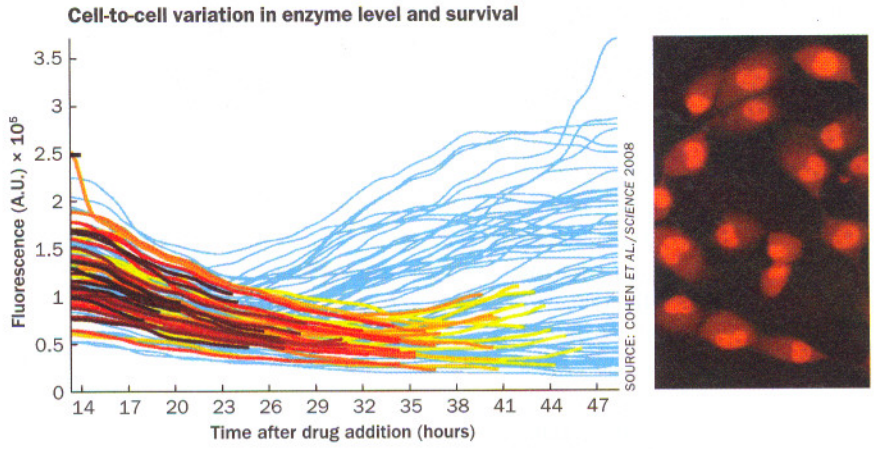
"I wouldn't consider a few theoretical papers as a substantial claim unless it's backed up by experiments," Xie comments. "That's my experimentalist prejudice."

Grima acknowledges that this is a weakness of his argument. "At this point, what is limiting its more wide acceptance is combined theoretical and experimental studies," he says. "Nobody has done a study in which they do a model, make predictions and then test them, all at one go. This would be the killer."

Such an experiment may be around the corner. At the moment, there are limited techniques for getting quantitative data on individual molecules inside a cell without killing the cell in the process. But several groups — including Xie's — are developing more. A recent review paper in *Trends in Biotechnology* heralds single-cell analyses as a new frontier that will transform differences between cells "from a source of noise to a source of new discoveries."

Even if future observations of the location and concentration of enzymes vindicate Michaelis and Menten, though, many scientists think that the emerging street-level details of the cellular city will continue to challenge traditional ideas.

"Any reaction occurring inside the cell will be impacted by these conditions, but we don't know exactly how," Grima says. "We're probably sitting on top of the iceberg." ■



Life or death Differences in enzyme levels among individual cells were tracked using a red fluorescent marker (right). Human tumor cells (at left, each line represents one cell) that withstood a dose of the anticancer drug camptothecin made more of the DDX5 enzyme (as measured by fluorescence level), while enzyme levels dropped in the cells eventually killed by the treatment. Blue lines mark cells that survived treatment. Other colors show cells that died, with the darker colors corresponding to earlier cell death.

Lisa Grossman is a writer in Seattle.

Explore more

■ Daojing Wang and Steven Bodovitz. "Single cell analysis: the new frontier in 'omics.'" *Trends in Biotechnology*, June 2010.