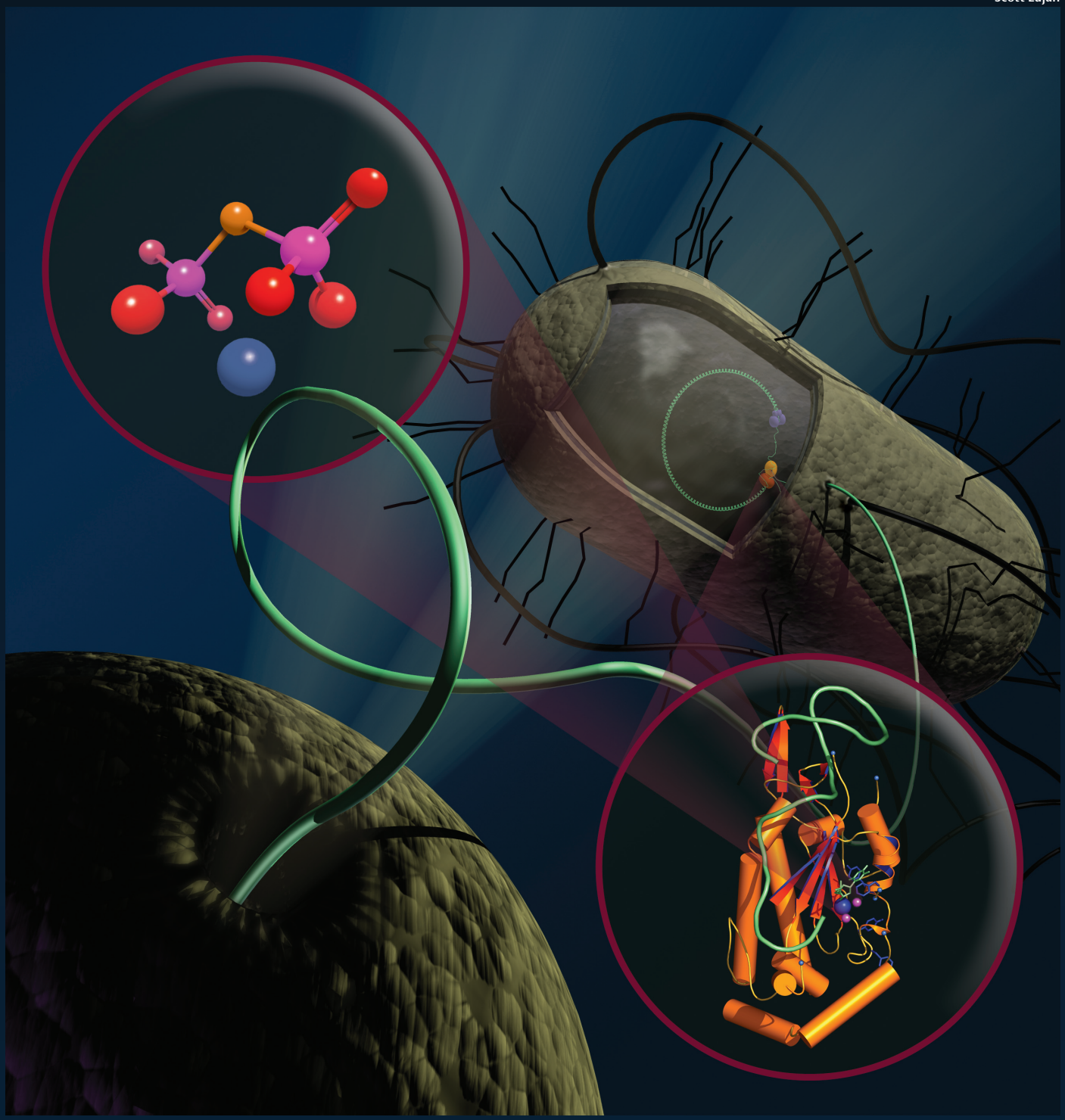
Scott Lujan



If a bacterium has resistance to antibiotics, it can donate that resistance to other bacteria by copying DNA strands that contain the resistance genes. UNC scientists have discovered that drugs called bisphosphonates *(inset top left)*, already approved to treat bone loss, can stop the transfer of antibiotic resistance genes. They can even selectively kill bacterial cells that harbor resistance.

the bug zappers

by danielle jacobs

Bacteria are getting it on. Unfortunately, some of them are also passing on antibiotic resistance. But Matt Redinbo and Steve Matson say they can put an end to that.

Bacteria are pretty simple. They have no secret compartments. They're just tiny bags of stuff, mostly chromosomal DNA and plasmids. Chromosomal DNA is necessary for bacteria to live. Plasmids are not. They're simply long, circular coils of DNA that broke free from the chromosome at some point in the bacteria's evolution. But plasmids carry genes that can encode for such traits as antibiotic resistance. They can also replicate and donate themselves—and their resistance—to other bacteria.

Plasmids are copied and carried to other cells by a mechanism called conjugation. (Imagine sharing a stick of braided licorice: grab a strand, tear it apart from the rest, and give it to a friend.) DNA has two double-helix strands—one called Watson, the other Crick (they're named for the scientists who first described them).

To conjugate, the *E. coli* bacterium uses three enzymes. First a relaxase grabs onto Watson and breaks it. Next, while the relaxase is still holding on, a helicase unravels Watson from Crick. A replisome bursts in while the strand is unwinding; it shadows and copies Watson, replacing it with an entirely new one.

After the helicase has gone around one complete circle of the plasmid's coil, the relaxase lets go of Watson, setting it free into another bacterial cell. Once there, Watson is replicated and reunited with his old friend Crick once again.

And if Watson had antibiotic resistance, now the new cell has it, too.

Matson has been studying bacterial conjugation in *E. coli* since the early 1980s, when Redinbo was still in high school. Matson decided to study TraI, which scientists had identified as a helicase—the enzyme responsible for unraveling the plasmid. But in 1991 Matson and his lab found that TraI was a lot more talented than anyone thought. It carries both the helicase *and* the relaxase—something that had never been seen before. TraI has all the tools to grab, unwind, and release a strand into a new cell.

Matson now had even more questions to answer, but he wasn't intimidated. He wanted to learn more about the relaxase. But even after he isolated and purified the relaxase portion of TraI, he couldn't see how it worked. He sought out Matt Redinbo, now a biochemist with a track record for crystallizing complex proteins (essentially taking a still photo on an atomic level).

But getting a good picture of the relaxase wasn't easy. It took Redinbo's team three years to get the shot. And just a few months before they got it, a group in Baltimore got it first—a glamour shot of the enzyme all alone, primped for its moment in the spotlight.

But while getting a snapshot of an enzyme by itself is good, catching it in the act is even better. Scott Lujan, a graduate student in Redinbo's lab, crystallized the relaxase doing its business with DNA, which finally allowed the chemists to understand how TraI grabs and releases a single strand of plasmid DNA.

After decades of learning how TraI works, Matson and Redinbo wanted to figure out how to stop it.

Knowing that conjugation in *E. coli* is dependent on a well-functioning relaxase, the biochemists believed they could inhibit conjugation—and thus the transfer of antibiotic resistance—by making the relaxase bind with something that merely *resembled* the plasmid. DNA's strands—Watson and Crick—are made up of sugars and phosphates. Bisphosphonates are molecules that are made up of two phosphates. Lujan used the simplest bisphosphonate he could find, hoping to confuse TraI so that it couldn't interact correctly with the plasmid.

And it worked. It worked so well that the *E. coli* donor cells *died* before they could even attempt to share their goods with anyone else.

The results were so surprising, Lujan says, that he "actually did that first experiment eight times." But when they brought in other bisphosphonates, including two simple drugs already on the market for osteoporosis, they saw similar effects. "We finally convinced ourselves that it was real," Lujan says.

Redinbo and Lujan think that the cells die after the relaxase tears apart the two strands and the helicase starts to unwind them. The bisphosphonate prevents the relaxase from letting go of the unwound strand. Instead, Lujan says, "it just keeps unwinding and unwinding around and around, until it uses up all of the cell's resources." The cell dies, leaving behind only pieces of Watson and Crick.

But Matson and Redinbo only studied this relaxase inhibition in *E. coli* bacteria—which despite their reputation, are relatively benign: right now, there are billions of them in your lower intestine. The *E. coli* F plasmid,

Lujan says, "was the first one ever discovered, so it's been the most intensely studied and made a good model system." Now Redinbo's lab is certified to bring in nastier, meaner bugs to test: harmful bacteria such as *Pseudomonas* and *Staphylococcus*, which often have plasmids that encode for antibiotic resistance. As of now the team's bisphosphonates don't appear to be picky about the plasmids they work with—and when it comes to killing antibiotic-resistant bacteria, that's a good thing.

Multi-drug resistant infections such as MRSA—a kind of staph bacteria that's resistant to the antibiotic methicillin—are, ironically, most often picked up at the hospital. Redinbo foresees his team's inexpensive bisphosphonates being a first line of defense against these infections. "If you're going in for standard surgery," he says, "and somebody on the ward has a resistant infection, you could give this as a preventive medication, especially for the elderly or young."

And for patients who are already sick, Lujan says, the worst thing you can do is loosely dispense more antibiotics. Instead he envisions bisphosphonates used in hospitals to treat resistant infections *prior* to antibiotics.

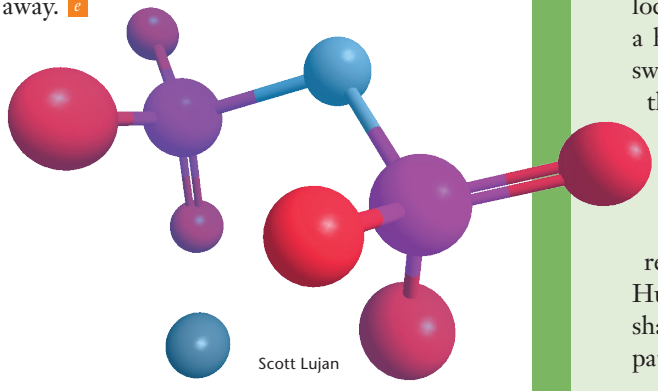
"One thing to understand is that none of the bisphosphonates are particularly good antibiotics," Lujan says. "A good antibiotic will wipe out 99.999 percent of the bacterial population. The best we've been able to do is 99 percent."

So instead of trying to kill all of the bacteria in one fell swoop with an antibiotic, inevitably leaving resistant stragglers behind, Lujan suggests fighting bacteria with a one-two punch: hospitals could first administer bisphosphonates to an infected patient, wiping out the cells containing antibiotic-resistant plasmids. Once those cells are all dead—the team has seen this in as little time as an hour and a half—doctors could then hit the patient with a standard antibiotic, which should effectively kill off the rest of the bacteria.

> "If you're going in for standard surgery, and somebody on the ward has a resistant infection, you could give this as a preventive medication, especially for the elderly or young."

But even if it doesn't, at least the few left won't have any resistance to give away.

Steven Matson is a professor in and chair of the Department of Biology. Matthew Redinbo is a professor in the Departments of Chemistry, Biochemistry, and Biophysics, and a member of UNC's Lineberger Comprehensive Cancer Center. Scott Lujan is a post-doctoral fellow studying biochemistry in the School of Medicine.



Scott Lujan

tiny steps on a tightrope

Tracking down how proteins have evolved is something of a detective game.

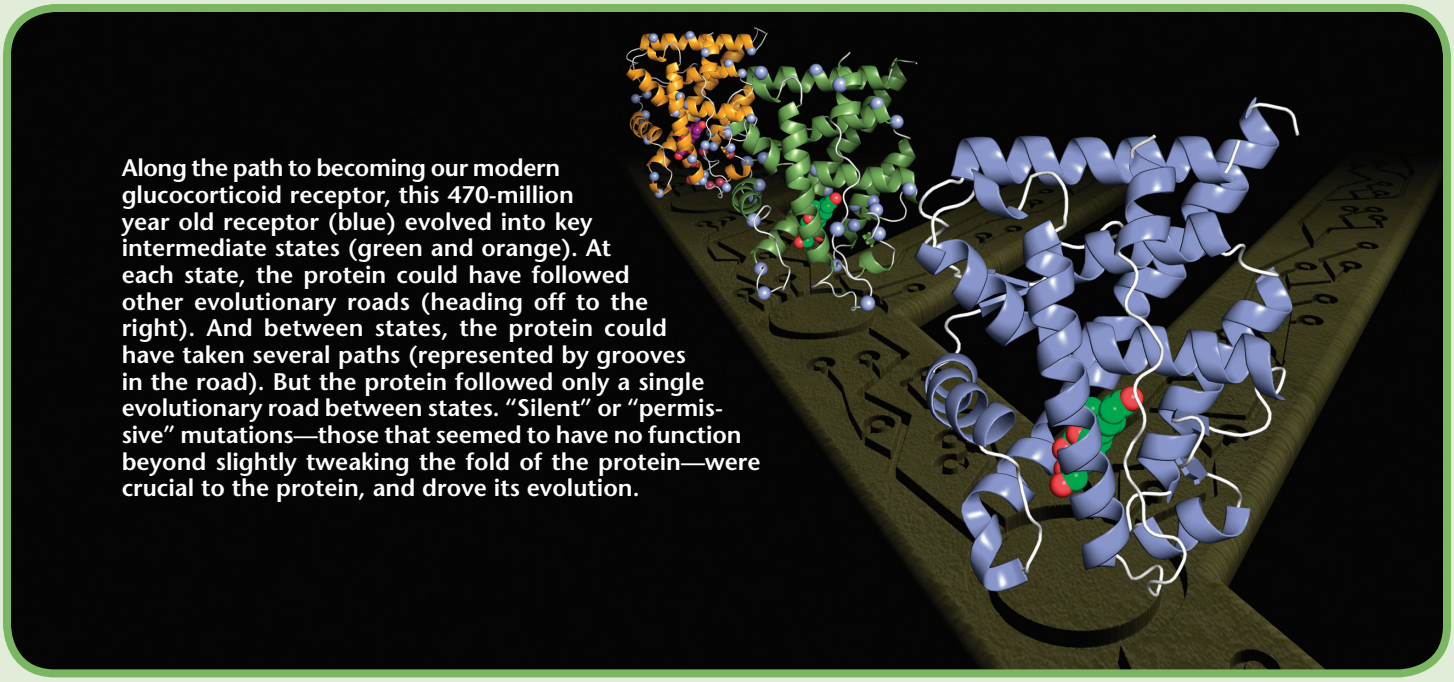
Historically, scientists have worked backward in time using educated guesswork and knowledge of protein chemistry to figure out the probable evolutionary routes that proteins took to their modern form. Until recently, that is.

Matt Redinbo and his colleagues decided to start near the beginning. Redinbo and Joseph Thornton at the University of Oregon used crystallography to create the first map of the exact structure of an ancient protein. Then they virtually strolled down the evolutionary path taken by the protein more than four hundred million years ago.

Organisms typically get more complex as their proteins evolve new functions. To do that, proteins must undergo mutations that change their structure. Some of these mutations are clunky and break the protein, but others are vital and endow the protein with new traits. To identify which historical mutations were vital, Redinbo's team compared proteins to their precursors through evolutionary time.

They reconstructed the ancestor of two hormone receptors, which function like locks in a lock-and-key mechanism. When a hormone binds a receptor, the receptor switches on a cascade of signals that allows the cell to perform a specific function. Redinbo's ancestral protein ultimately evolved into the stress hormone receptor, or the glucocorticoid receptor (GR), and the mineralocorticoid receptor, which controls kidney functions. Humans and bony fish have both receptors; sharks, which took a different evolutionary path, have only the latter.

Eric Ortlund



Along the path to becoming our modern glucocorticoid receptor, this 470-million year old receptor (blue) evolved into key intermediate states (green and orange). At each state, the protein could have followed other evolutionary roads (heading off to the right). And between states, the protein could have taken several paths (represented by grooves in the road). But the protein followed only a single evolutionary road between states. "Silent" or "permissive" mutations—those that seemed to have no function beyond slightly tweaking the fold of the protein—were crucial to the protein, and drove its evolution.

"This wasn't like Jurassic Park where there was a sample drawn out of a fossil," Redinbo says. "We statistically determined the sequence of the hormone receptor's ancestor from an organism that lived over four hundred million years ago in the deep oceans. You can think of this creature as the last common precursor of a sea bass and a human."

Once the team had determined the sequence of the ancient receptor, they engineered bacterial cells to produce the ancestral receptor by injecting the gene corresponding to its sequence into the bacteria. The reconstructed receptor was stable and functioned normally, which confirmed that their statistical predictions were correct.

What they did next gave them unique insight into how the protein evolved. Using a biophysical technique called X-ray crystallography, they pinpointed the precise location in three-dimensional space of every single atom in the reconstructed receptor. Redinbo's team bombarded the crystals of the ancient receptor with X rays, which bounced off the crystals. The X rays were then exposed to a film, where they formed a blueprint of all the atoms in the receptor.

The team then compared the ancestral receptor to the modern GR found in humans, and looked for mutations that changed the former into the latter. Five specific mutations stood out. These seemed to be responsible for the new function that the ancient receptor had evolved: specificity to the stress hormone cortisol.

Next the team engineered those five mutations into the ancient receptor in hopes of creating the modern GR. To their surprise, the new protein fell apart. They were either completely on the wrong track, or they were missing a vital link.

On closer examination, the team found two other mutations that happened by chance earlier in the evolutionary timescale. These two mutations didn't seem to have any function other than slightly tweaking the fold of the protein. The new fold, though, turned out to be pivotal in strengthening the structure that the five later mutations dictated.

"It's like cutting a window in your house and having your whole house fall down," Redinbo says. "But if you were to alter the floor of the house just a little bit, then that new window would work out and change the way the house functions." And that's what they discovered: without the two silent mutations, the five specific mutations made the protein unstable.

It's as if the ancestor were walking an evolutionary tightrope toward its new function, Redinbo says. "As random mutations kept happening, those that destabilized the ancestor would cause it to fall off and were therefore thrown out, but those that added zero value to it were not always eliminated. The two mutations that survived turned out to be essential for the five later ones that gave the ancestor a completely new function. We think that such permissive mutations may underlie the new functions taken on by most proteins in their evolutionary history."

Evolutionary forces are intimately tied to cancer and other diseases, as well as resistance to antibiotics, says Eric Ortlund, first author of the study and a former postdoc in Redinbo's lab. "One could certainly accelerate evolution in the lab to understand how proteins may circumvent inactivation by drugs while preserving their function," he says.

"This wonderful piece of structural biology fills a big gap in the great conceptual puzzle of how proteins adapt to new functions," says Ichiro Matsumura, an evolutionary biologist at Emory University. "I expect it will take time for Redinbo's ideas to percolate through the scientific community. But that is the price one pays for thinking ahead of the curve." e

—Prashant Nair

Prashant Nair is a master's student in medical journalism at Carolina.

Matthew Redinbo is a professor in the Departments of Chemistry, Biochemistry, and Biophysics, and a member of UNC's Lineberger Comprehensive Cancer Center. His team was funded by the National Institutes of Health, the National Science Foundation, and by a Lineberger Comprehensive Cancer Center Postdoctoral Fellowship.