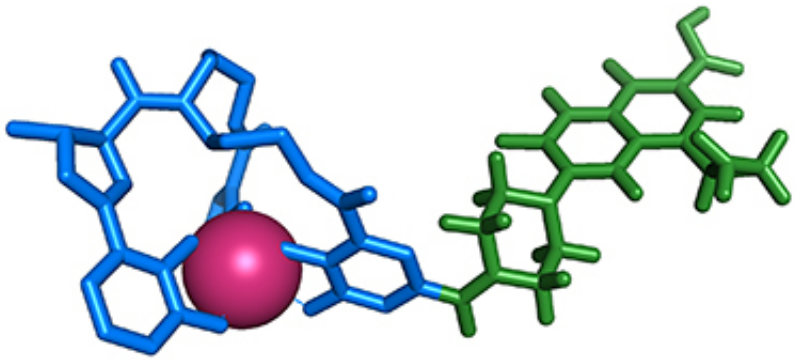
Slaying bacteria with their own weapons

June 26 2014



A hypothetical Trojan Horse drug would consist of a siderophore (blue) linked to an antibiotic, in this case the broad-spectrum antibiotic ciprofloxacin (green). Many bacteria have evolved resistance to ciprofloxacin in recent years, leaving it less effective than it once was. Attaching it to a siderophore might make the antibiotic useful again because bacteria pump siderophores inside their cell membranes where they can do maximum damage. Credit: generated using PDB file 4K19 (Allred, B. E.; Correnti, C.; Clifton, M. C.; Strong, R. K.; Raymond, K. N. ACS Chemical Biology 2013, 8, 1882-1887

The Centers for Disease Control and Prevention warned last fall that the U.S. faces "potentially catastrophic consequences" if it doesn't act quickly to combat the growing threat of antibiotic-resistant infections, which kill about 23,000 Americans a year.

Timothy Wencewicz, PhD, assistant professor of chemistry in Arts &

Science at Washington University in St. Louis, has an idea that might provide a solution to resistance that is broader and more effective than the invention of a new drug.

"Today when you walk in with the symptoms of a bacterial infection," Wencewicz said, "you are treated with a broad-spectrum antibiotic because we lack the ability to identify a bacterial strain quickly. And that means every type of bacteria in your body is exposed to that antibiotic." So even justified antibiotic use that follows medical protocols encourages resistance.

One solution, he said, is personalized antibiotic therapy. This would require both rapid bacterial identification and narrow-spectrum antibiotics. Tailored antibiotic therapy would not only extend the clinical lifetime of new antibiotics by better managing resistance, it might also revive old antibiotics that have been abandoned due to resistance, toxicity, or their inability to penetrate bacterial membranes.

Wencewicz is working on a drug delivery system that would target specific bacteria by exploiting small molecules called siderophores they secrete to scavenge for iron in their environment. Each bacterium has its own system of siderophores, which it pumps across its cell membrane before cleaving off the iron.

If an antibiotic were linked to one of these scavenger molecules, it could be converted into a tiny Trojan horse that would smuggle antibiotics inside the bacterium's cell membrane. Not only would the bacterium be directed against a specific pathogen, it would be effective at much lower concentrations because it would have penetrated the bacterium's outer defenses.

What's more, because each bacterial species has its own siderophore system, these molecules and their receptors could be used to rapidly

identify the bacterial strain causing an infection. Siderophore diagnosis could be paired with siderophore drug delivery to provide personalized therapy that would spare patients trial-and-error treatment and, more importantly, make it much more difficult for bacteria to develop resistance.

Wencewicz has received a Ralph E. Powe Junior Faculty Enhancement Award from Oak Ridge Associated Universities (ORAU), a 114-member university consortium whose mission is to advance scientific knowledge, to systematically investigate a panel of 25 siderophores he synthesized while he was a graduate student at the University of Notre Dame. Using a laboratory strain of *Staphylococcus aureus* as a bioassay to test which ones work best, the goal is to lay the basis for rational siderophore-antibiotic drug design.

So, it is to be war between us!

With billions of years of evolution at their backs, bacteria have developed mind-boggling survival skills. One of these is finding iron even in environments where it is present in insoluble forms or at very low concentrations.

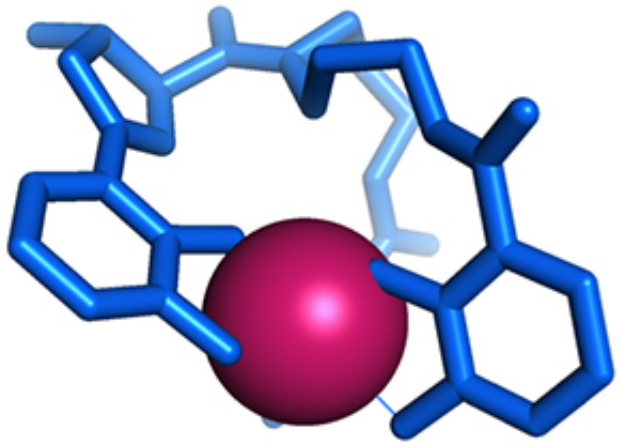
Iron, Wencewicz explains, is essential for life and plays a role in many biological processes, including oxygen transport. Although iron is one of the most abundant elements in the Earth's crust, most of it is in the iron(III) state—the most stable form of iron in an oxygenated environment—which is very insoluble and so essentially bio-unavailable.

"Bacteria have to scavenge the nutrient directly from insoluble iron minerals in the soil. And to do that they've evolved the ability to make these fascinating molecules called siderophores," he said. Siderophores are iron chelators (from the Greek khele meaning "having pincer-like claws.") "They grab onto iron, pull it off of the insoluble source and

bring it into aqueous solution." (See the video, above, for a demonstration.)

Because bacteria are competing for iron, Wencewicz said, there is pressure to evolve a siderophore no other bacterium can recognize and steal. More than 500 are known, but Wencewicz thinks there are probably tens of thousands that haven't yet been found. Each bacterium has receptors in its cell membrane that recognize its own siderophore, which it then pumps inside its membrane.

Sometimes this works and sometimes it doesn't. "Bacteria are very clever, " Wencewicz said, and "they've evolved to steal siderophores from other bacteria by stealing their DNA and expressing the protein coded by the DNA. There are bacteria out there that maybe make only one siderophore, but they can recognize 10 and transport them across their cell membranes. They make their buddies do the work."



A siderophore called fluvibactin made by the bacterium *Vibrio cholera* (blue structure) that has bound an iron atom (pink sphere). Credit: generated using PDB file 4K19 (Allred, B. E.; Correnti, C.; Clifton, M. C.; Strong, R. K.; Raymond, K. N. ACS Chemical Biology 2013, 8, 1882-1887).

Bacteria also confront a hostile iron-poor environment when they invade our bodies. Free iron is toxic so almost all of the iron in our bodies is bound to proteins, such as hemoglobin (in red blood cells) and the less familiar ferritin (inside cells) and transferrin (circulating in our blood).

So once insider our bodies, infecting bacteria make and secrete their siderophores, looking for iron. Some of them can also pry open our iron-storing proteins and break into cells to get at the iron sequestered there.

We counter the siderophore onslaught by making molecules called siderocalins that bind siderophores, preventing them from binding iron. But some bacteria sidestep this defense, making a sacrificial siderophore that saturates the available siderocalin and a second "stealth" siderophore that siderocalin doesn't recognize.

"Each pathogen is unique," Wencewicz said. "If we are going to use its own siderophores against it, we have to understand each siderophore it secretes, its function, and how its function is related to its structure.

"We have to quit thinking that every single bacterium is like all the other bacteria. They're not," he said.

Beware of Greeks bearing gifts

Wencewicz' central insight is that this specificity—and the fact that the bacteria bring siderophores inside their cell membranes—can be turned against them.

He is taking his cue from the bacteria themselves. Some bacteria, he said, link a toxic antibacterial agent to a siderophore, creating something called a sideromycin.

Competing bacteria, attempting to steal iron, grab the sideromycin and shuttle it in through their siderophore-uptake system. Once the sideromycin is inside the bacterium, the toxin it carries kills the bacterium.

Because the siderophore system is highly specific, Wencewicz believes it can be used to target even broad-spectrum antibiotics to individual bacterial strains, avoiding the indiscriminate exposures that spread resistance. And because the Trojan horse antibiotics will ferry antibiotic inside the membrane, only tiny doses will be needed.

For both reasons he believes siderophore delivery systems will delay the emergence of resistance to new antibiotics and rescue many older antibiotics that have been abandoned because of resistance or toxicity.

Targeted therapy would be useless without rapid diagnostics because doctors wouldn't know which sideromycin-like drug to prescribe. But because siderophore systems are specific to bacterial species, they could be used to type as well as to treat bacteria.

Because of resistance, we really need to discover new approaches to antibiotic development and not just continually re-jigger existing drugs, Wencewicz said. The siderophore pathway offers the opportunity to completely re-imagine antibiotic development so that it takes resistance into account up front instead of on the back end.

Provided by Washington University in St. Louis